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JULY, 1933

Phytopathology

An International Journal

Official Organ of
The American Phytopathological Society

CONTENTS

Roland Thaxter (1858–1932), his influence on plant pathology. Wm. H. Weston, Jr. 565

A cytological study of the teliospores, promycelia, and sporidia in Puccinia malvacearum ..... Ruth F. Allen 572

A new Sclerospora from Nyasaland ..... Wm. H. Weston, Jr. 587

Stem canker of Crotalaria spectabilis caused by Diaporthe crotalariae, n. sp. ..... George F. Weber 596

The pathology of tobacco black shank ..... Frederick Taylor Wolf 605

Field observations of needle rusts of spruce in Minnesota. Ralph M. Lindgren 613

Consistent mutation of Helminthosporium sativum on a no-nitrogen medium ..... Glenn E. Paxton 617

Phytopathological Notes:
Three new hosts for Tylenchus dipsaci, the bulb or stem nema. G. Steiner and Edna M. Buhrer
Unusual disease symptoms produced by Aphelenchoides fragariae ..... G. Steiner and Edna M. Buhrer
A potato disease ..... Donald Reddick
Book review 625

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Official Organ of The American Phytopathological Society

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DR. THAXTER ON HIS 70TH BIRTHDAY IN HIS GARDEN AT KITTERY POINT, MAINE, WHERE IN LATER YEARS HE STILL CONTINUED HIS INVESTIGATIONS OF PLANT DISEASES.

PHYTOPATHOLOGY

VOLUME 23 NUMBER 7

JULY, 1933

ROLAND THAXTER (1858–1932), HIS INFLUENCE ON PLANT PATHOLOGY

WM. H. WESTON, JR.

Although known primarily for his work in mycology, none the less Dr. Thaxter exerted a distinct influence on plant pathology, not only directly through his own brief but outstanding work in this field, but also indirectly, through the inspiring example of the high standards of accuracy and completeness he maintained throughout all his investigations, through the aid in difficult mycological problems he gave to the many perplexed pathologists who consulted him, and through the training and ideals he transmitted to students who have since become important factors in phytopathology.

He was born on August 28, 1858, and naturally during the period of his school and college training he received little or none of the specialized and intensive instruction in plant pathology which is offered in the famous phytopathological laboratories of the present. It must be remembered that he entered Harvard from the Joshua Kendall School in Cambridge in 1878 only a few years after Asa Gray, with progressive foresight, had secured the appointment of G. L. Goodale in Plant Physiology and W. G. Farlow in Cryptogamic Botany. Consequently Dr. Thaxter's college course, from which he received in 1882 his A.B. degree, Magna Cum Laude, with honorable mention in Natural History and English Composition, gave him a sound background in botany and zoölogy rather than any specialized training in plant pathology. Indeed, it was not until 1884 that he began to concentrate in botany, especially mycology, when, after a year in the Medical School, he started graduate work with Dr. Farlow under two years' tenure of the Harris Fellowship.

His first mycologic paper, "On Certain Cultures of Gymnosporangium with Notes on their Roesteliae,"[1] published in 1887, was of pathologic as well as mycologic significance, since by culture studies and cross inoculations he demonstrated the relation of several species of Gymnosporangium to various Roesteliae of the vicinity. Realizing the difficulties involved, the plants of various species of Crataegus, Pyrus, and Amelanchier, potted in April before the leaves developed, had been kept in four separate rooms in Boston some miles from any cedar trees to avoid any possibility of outside

[1] For a list of Dr. Thaxter's publications see Weston, Wm. H. Roland Thaxter. Mycologia 25: 69–89. 1933. (Publications, pp. 86–89).

infection, and to these from the seven common local species of Gymnosporangium, similarly forced to an early production of telial columns, cross inoculations were made which definitely established the connection with the roestelial stages in five of the seven cases. Moreover, appreciating the possibility that sexuality and fertilization might be involved in the development of aecidia, he attempted to fertilize female organs that might be present by placing the spermatia on the under side of the leaves of the Rosaceous hosts, and although his results were not conclusive, it should be noted that he foresaw this possibility which only recently has been demonstrated as an actuality in other rusts. It should be noted also that in this connection he pointed out the possible rôle of flies in achieving such fertilization in nature, a shrewd suggestion probably derived from his thorough familiarity with the Diptera, for even at this time he had an unusual knowledge of insects and had published several entomological papers.

With characteristic tenacity he did not drop this investigation when he went on into other fields, but even while working on his thesis, "The Entomophthoreae of the United States," for which he received his Ph.D. in Natural History in 1888, he continued his study of cultures of Gymnosporangium, using a greenhouse under more carefully controlled conditions than had been possible previously. Although his procedure would now be considered heretical, as he did not use a series of plants as controls, none the less his precautions in forcing both the Gymnosporangium and the hosts so that the inoculations were made in the greenhouse a month or so before any infection occurred in nature, gave security to his results and enabled him in this paper of 1889 to corroborate his previous work, to secure in addition the roestelia of *Gymnosporangium globosum,* and by careful comparison with authentic herbarium material to establish with certainty the identity of the aecial stages which he had developed. These two papers were a worthy continuation of Farlow's "Gymnosporangia or Cedar Apples of the United States," ("cider apples," according to one biographer!) of 1880, and "Species of Gymnosporangium and Chrysomyxa" of 1885, and furnished convincing answer to the hope expressed by Peck in the Botanical Gazette of 1881 for a continuance of such investigation "until . . . the supposed connection between the Gymnosporangia and Roesteliae shall be shown to be either real or imaginary."[2]

In 1888, after receiving coincidentally both M.A. and Ph.D. degrees from Harvard, he went to his first position, that of Mycologist at the Connecticut Agricultural Experiment Station, which, as the 1907 year book of his class of 1882 takes pains to point out, "although located in New Haven, has no connection with Yale." His annual reports and other publications

[2] Peck, C. H. [Review of] The Gymnosporangia or cedar-apples of the United States, by W. G. Farlow. Bot. Gaz. 6: 209–210. 1881.

during the three years he worked in this capacity give convincing evidence in their "miscellaneous notes," of his unusual knowledge of fungi and his wide-ranging activities as a mycologist, and, in their detailed reports on important diseases, of his effectiveness as a plant pathologist. His work on the diseases of onions and particularly his reports on onion smut were valuable contributions to plant pathology, covering thoroughly the historical background and the pertinent literature, furnishing important information on the distribution, dissemination, and effects of the disease, working out in careful detail the structure, development, and methods of infection of the causal fungus, and giving practical, simple, and inexpensive methods of prevention and control based on careful experiments. Although decidedly mycological in point of view, the work is distinctly in accord with the traditions of present phytopathological procedure, comprising convincing demonstrations and illustrations of treated and untreated rows in experimental plots, and making effective use of information secured by means of questionnaires sent to persons and firms interested in onion cultivation throughout the State. Additional notes on the mildew, the Vermicularia, and the Macrosporiums of onion, as well as lists of fungi parasitic on the genus Allium, help to add to the completeness and thoroughness of this pioneer report, which still stands as significant in its field.

However, it is perhaps his study of potato scab that is the best known of his phytopathological work while at the Connecticut Station. His investigation of this disease, his thoroughgoing discussion of its history and of the theories as to its origin, his careful and convincing mycologic work in separating the secondary fungi, isolating the causal organism and proving its pathogenicity, make this one of the most interesting and important of his plant pathological papers. The work on potato scab he continued during a second season, confirming by experiments his previous conclusions, answering conclusively the doubt of other investigators as to his results, and in the preventive measures that he had found advantageous, emphasizing especially the importance of using seed free from scab.

That this young investigator, primarily a mycologist in training and inclination, with ready adaptability could turn all the resources of his training, his knowledge, and his natural ingenuity to the practical problems of the control of plant diseases, was well demonstrated also in his work on the leaf spot of quince, which at that time had become so serious in Connecticut that some orchards were of necessity being abandoned. Enlisting the cooperation of some of the growers, Dr. Thaxter made careful experiments in spraying with fungicides through parts of their orchards, leaving other parts untreated as controls. For this work he devised a spraying outfit, consisting of a copper wash boiler with a hand force pump and a Vermorel nozzle, and this apparatus, although costing only a little over eight dollars,

and crude in comparison to the improved modern types, proved superior to the Eureka sprayer owned by the station, and is of interest to us now as one of the earliest outfits used in the pioneer days of plant-disease control by fungicides in this country. The work on controlling leaf spot of quince, which he carried on through the seasons of 1890 and 1891, demonstrated conclusively that from a single treated row could be secured a net income one hundred times as great as that from an untreated row, and on the practical basis of such impressive balance of yield over expense, went far toward converting even the most hard-headed and unprogressive growers of that time to the then "new-fangled" practice of preventive spraying. Although the major part of his investigation was concerned with quince leaf spot, he tried out various fungicides on black rot of grapes as well, and, by interesting progressive farmers and growers in coöperative spraying experiments, secured very satisfactory results with leaf spots of plums and cherries, and with other diseases such as late blight of potato. His reports on spraying and the use of fungicides in plant-disease control took up in detail the suitability of various fungicides, the best methods and times of application, the requisite features of the apparatus, the costs involved and the yields secured.

While at the Connecticut Station also, he described *Phytophthora phaseoli*, a new species on Lima Bean, from the pathologic point of view discussing its destruction, distribution, method of spread, and effect on the host, and from the mycologic point of view examining its distinguishing characteristics and establishing it as a new species. It is a tribute to his mycologic knowledge and discernment that this species had retained its specific rank through the recent critical physiologic, biometric, and cultural reinvestigations in which so many species have been relegated to synonymy.

Shorter papers, both the continuation of his work on Gymnosporangium in "The Connecticut species of Gymnosporangium (cedar apples)" and his study of the fungus in violet roots, *Thielavia basicola*, although short notes, show the same characteristic care and thoroughness in investigation.

He was mycologist at New Haven for only three years (1888–1891) but in this time he contributed definitely to the development of plant pathology. His work showed a sound mycological background, a wide knowledge of the pertinent literature, a flair for convincing experimentation, a ready ingenuity and adaptability coupled with conservative persistence and exacting accuracy. As Clinton,[3] in his vice-presidential address on "Botany in Relation to Agriculture" has pointed out, Dr. Thaxter advanced plant pathology materially by important work on the artificial culture of pathogenic fungi and by pioneer contributions to plant-disease control through preventive spraying for quince leaf blight, seed selection from potato scab,

[3] Clinton, G. P. Botany in relation to agriculture. Science n.s. 43: 1–13. 1916.

and sulphur treatment for onion smut. Yet, through all his earnest devotion to his work he retained his characteristically dry sense of humor, for in his copy of the report of an annual agricultural convention in 1888, in the announcement "The quartet again favored the audience with a song, after which the Chairman introduced as the lecturer of the afternoon, Dr. Roland Thaxter" he starred the word "song" and at the bottom of the page added in his precise handwriting, "*Entitled "Oh Spare me! Spare me! Free me from this pain!"

Moreover, during these years, although carrying on pathological work that would have overwhelmed many men, he still found time to continue purely mycological investigations which led to descriptions of interesting new genera and species of the Imperfecti in two papers on "Certain new or peculiar North American Hyphomycetes" in 1891, and through successful culture of a Chondromyces parasitic on lichens around New Haven revealed the true nature of this organism on which as a basis he later established the Myxobacteriaceae as a new order.

In 1891, the period of Dr. Thaxter's direct contributions to phytopathology ended and that of his indirect influence as a teacher began, for he was called back to Harvard to be assistant professor associated with Dr. Farlow. Active in research and in teaching, he was given ever-increasing responsibility, as Dr. Farlow in 1896 gave up the instruction of undergraduates; and from 1901, when he became full professor, he carried the full burden of Cryptogamic Botany until after Dr. Farlow's death in 1919 he became Professor Emeritus and honorary Curator of the Farlow Library and Herbarium, voluntarily retiring to devote his remaining years to his investigation of the Laboulbeniales.

During these years nearly a thousand students attended courses under Dr. Thaxter or worked with him as graduate students for higher degrees. Although these courses, the more elementary in Cryptogamic Botany and the more advanced ones devoted to the fungi, algae, and bryophytes, gave no specific intensive training in plant pathology as such, none the less they inculcated ideals of exacting accuracy and painstaking thoroughness, furnished an indispensable background, and gave much directly valuable training and information on culture methods, life histories, and measures of prevention or control. It is true that of the men especially trained for their life work by studying as graduate students under Dr. Thaxter, only a relatively small proportion devoted themselves to plant pathology as a profession, yet from this number between 20 and 30 have since been influential, ranging from some of his earlier students such as J. B. Rorer and J. R. Johnston, whose names are known in tropical plant pathology, and G. P. Clinton, H. P. Barss, H. S. Jackson, and J. H. Faull, active in plant pathol-

ogy in the United States and in Canada, through R. H. Colley, W. A. McCubbin, and O. F. Burger of more recent years, down to Charles Drechsler and F. C. Meier among the last of the men trained by him before he relinquished teaching in 1919.

His indirect influence on plant pathology, however, was not exerted solely through his training such able men as these. His extensive correspondence furnishes ample evidence of the invaluable assistance he gave the many pathologists who, recognizing his extraordinary knowledge of the fungi, turned to him for help when in doubt over the identity or behavior of obscure or perplexing forms. Moreover, the high standards of accuracy, thoroughness, and completeness which he maintained throughout his own work, and upon which he insisted in the work of his students, unquestionably exerted a beneficial influence not only on mycology but on plant pathology as well.

In his later years Dr. Thaxter seems to have gained the reputation of being rather critical of plant pathology. It should be remembered, however, that he was ever ready to commend and esteem work of high quality whether it was done in the field of plant pathology and applied mycology or in the more restricted field of pure mycology. He had, it is true, a justifiable scorn for incomplete and inaccurate work, work lacking accuracy and thoroughness in investigation and experiment, deficient in familiarity with the pertinent literature, inadequate in necessary mycological background, and inferior in the quality of its presentation and illustrations. It was not that he deplored phytopathology as such, but rather that the very incompetence and complaisance with poor work which he decried seemed to him to be more prevalent in phytopathology than in the academic phases of botany. Even the famous "battle of Kittery Point," which has become a legend where mycologists and plant pathologists gather, was but an active expression of this same insistence on high standards, a natural impatience that in a campaign toward plant-disease control, involving complex biological problems, power should be vested in those without adequate training or knowledge to administer that power to its best ends. For the most part Dr. Thaxter's reputed scorn for phytopathology is the result of his dryly humorous allusions in such phrases as "squirt gun botanists" or "mycology of the stomach and the pocket book." As he himself had been a pioneer in the control of plant diseases by spraying, the irony of his phrase "squirt gun botanists" was obvious to himself as well as to plant pathologists, and when Clinton once sent him a Connecticut spraying calendar with an illustration of Dr. Thaxter's own hand-made washboiler spray outfit with the inscription "squirt-gun botanist," he enjoyed the joke keenly and hung the inscribed illustration on the wall among his curios. His allusions to "mycology of the stomach and the pocket-book" also were but

a humorous expression of his regret that suitable opportunities were so few that men with great potentialities for productive scholarship were forced into routine governmental or industrial positions. For example, when Dr. Lyman, after being the guiding spirit of so many of us who were fortunate enough to come under his stimulating influence at Dartmouth, had gone to the Federal Horticultural Board, Dr. Thaxter, in discussing possible openings with the writer, remarked with a sigh, "Of course one can always get some sort of position in Washington, and even able men like Dr. Lyman seem to like it there, I'm sure I don't know why!" He felt that it was a great loss when promising men were forced by economic conditions from the academic field of education and productive research, because it was his sincere conviction that teaching and research were the important and fundamental basis of a cultured and advancing civilization.

An outstanding scholar and investigator, an effective teacher, his nearly fifty years of devoted work exerted a significant influence far beyond his own sphere of endeavor, and his death on April 22nd, 1932, was a great loss not only to the field of mycology in which he has become internationally renowned, but to the related field of plant pathology as well.

LABORATORIES OF CRYPTOGAMIC BOTANY,
HARVARD UNIVERSITY.

A CYTOLOGICAL STUDY OF THE TELIOSPORES, PROMYCELIA, AND SPORIDIA IN PUCCINIA MALVACEARUM[1]

RUTH F. ALLEN[2]

(Accepted for publication August 20, 1932)

Puccinia malvacearum Mont., the rust of Althaea, Malva, and related genera, is the cause of a wide-spread disease and has been studied by botanists in many lands. According to Eriksson (12), the original home of the disease is either Chile, where it was found in 1852, or Australia, where its presence was recorded in 1857. It was found in Spain in 1869, in France in 1872, in England and Germany in 1873, in Belgium, Holland, Denmark, and Italy in 1874, and so on, throughout Europe.

Much of the early work on *Puccinia malvacearum* was devoted to learning its life history and mode of overwintering. Kellerman, in 1874 (21), recognized it as a short-cycle rust and traced its entrance into the host and the development of its mycelium. Plowright, in 1882 (31), followed the rust through successive telial generations and studied the formation of the promycelia. Both found that the rust overwintered as pustules, in sheltered spots.

According to Dudley (11), the rust appeared in America in 1886. Dandeno in 1907 (8), Taubenhaus in 1911 (37), and Hill in 1925 (16) studied the overwintering of *Puccinia malvacearum* in America, the latter concluding that "the immature teliospores of *Puccinia malvacearum* are able to withstand the severity of winter in Quebec (minimum temperature −35° F.) and germinate the next spring, producing infection of healthy plants."

The extensive work of Eriksson on *Puccinia malvacearum* in 1911 (12) and 1921 (13) was devoted to a proof of the mycoplasm theory, while that of Bailey, on this rust, in 1920 (2), consists of a refutation of that theory.

Robinson, in 1913 (33), studied the relations of *Puccinia malvacearum* to its host, the tissues invaded, and the effects of the fungus upon these tissues.

Sappin-Trouffy, in 1896 (35, 36), published extensive cytological studies in the Uredineae, including a brief account of *Puccinia malvacearum.* He found that the mycelial cells are uninucleate and the haustoria are variable, "*fourchus, spiralés, rameux, utriculaires, ou même pelotonnés.*" "*Le*

[1] Cooperative investigations between the California Agricultural Experiment Station and the Division of Cereal Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture.

[2] Acknowledgments are due the departments of Agronomy and Genetics of the University of California for courtesies extended to the writer during the progress of this investigation.

dévellopement ainsi que les phénomènes de fécondation [*i.e.*, nuclear fusion] *de la téleutospore sont les mêmes que dans les autres Puccinia.*" The teliospores germinate without a rest period, each cell giving rise to a curved promycelium into which the fusion nucleus migrates and then divides twice. If the teliospore is immersed in water the development is abnormal, the promycelium segmenting into conidia without forming sporidia.

While there have been a number of more recent cytological investigations of this rust, most of them, including the work of Blackman and Fraser, in 1906 (4), Olive, in 1911 (30), Werth and Ludwig, in 1912 (39), Moreau, in 1914 (28), and Lindfors, in 1924 (22), are concerned primarily with the mode of origin of the binucleate condition.

Jackson, in 1931 (19), in a discussion of the evolution of rusts, cites *Puccinia malvacearum,* a microcyclic form without pycnia, as a type probably homothallic, and Ashworth, in 1931 (1), presents as evidence of homothallism experiments showing that this rust, when grown in monosporidial culture, produces normal teliospores.

In all this work on *Puccinia malvacearum* little attention has been given to the nuclear details during the formation and germination of the teliospores. As the material is particularly favorable for such a study, the following account is presented.

MATERIAL AND METHODS

Teliospores of *Puccinia malvacearum* can germinate without a rest period, but, if conditions for germination do not occur, they retain viability for a considerable length of time. In July, at Berkeley, California, after a month or two without rain, the teliospores germinate with great rapidity and in great abundance. Hollyhock leaves, bearing infections, were washed and placed in Petri dishes lined with wet paper and the sori were fixed at half-hour intervals from 1½ to 5 hours thereafter.

For the study of the development of the teliospore, a young potted mallow plant was inoculated. A pad of wet paper was placed in a watch crystal; a rusted hollyhock leaf was placed on it and secured in position by rubber bands. A glass cylinder lined with wet paper was set over the plant and the watch crystal inverted and placed on it as a lid. A layer of wet paper was folded down about the top and secured by a rubber band. This placed the germinating teliospores directly over the plant in a small damp chamber. The plant was then heavily watered and placed under a greenhouse bench for 48 hours. Infections developed in abundance and material was fixed 10 to 12 days after setting up the inoculation, when the new crop of teliospores was developing.

The material was fixed in Flemming's medium and weak solutions and in chrom-acetic-urea. It was washed, dehydrated, imbedded in paraffin,

and sectioned. Safranin and methylene blue were used to stain the young teliospores. Iron-haematoxylin was found advantageous in studying the mature and germinating teliospores, as it left the heavy teliospore wall unstained and so permitted the study of its contents.

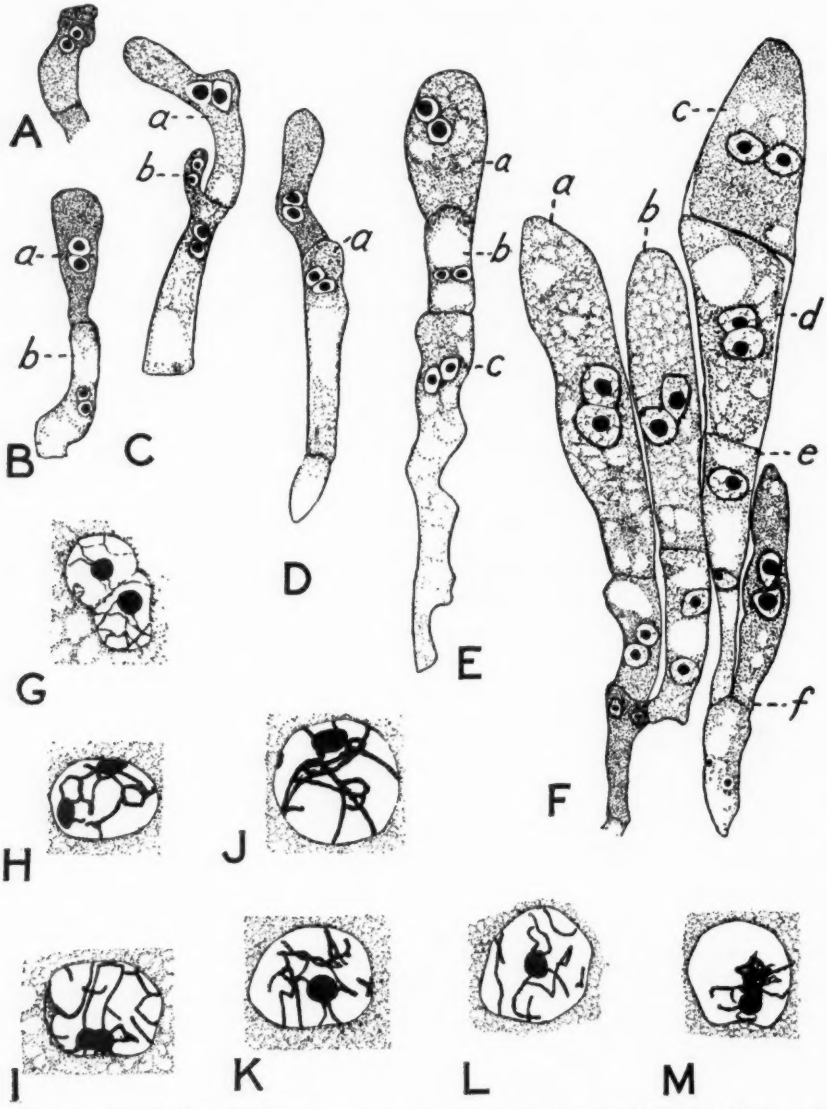

FIG. 1. *Puccinia malvacearum*. A to F, stages in the development of the teliospores; G to M, nuclear fusion and prophase of heterotypic division. A to E, ×1,020; F, ×1,400; G to M, ×2,250.

INVESTIGATIONS

Teliospores arise from binucleate cells. In figure 1, A, is drawn a young binucleate cell at a stage just before the initiation of spore formation. Nuclei and cytoplasm are concentrating at the distal end of the cell. It divides (Fig. 1, B) the terminal cell, *a*, receiving a pair of daughter nuclei and the bulk of the cytoplasm. This is the teliospore initial. The basal cell (Fig. 1, B, *b*) grows larger, its nuclei move to the apex of the cell (Fig. 1, D, *a*), divide, and a branch pushes out, which receives a pair of the daughter nuclei (Fig. 1, C, *b*). This is the beginning of a second teliospore. This branch soon becomes isolated from the parent by a septum (Fig. 1, F, *f*). If the sorus is well nourished this may be repeated, the same basal cell giving rise successively to 3 and even 4 teliospores. At this stage the teliospore initial is not infrequently bent (Fig. 1, C, *a*), being constrained into irregular form by the pressure of adjoining cells.

The teliospore initial grows and divides, the terminal cell (Fig. 1, E, *a*) becoming the spore and the second cell (Fig. 1, E, *b*) its stalk. The terminal cell becomes larger (Fig. 1, F, *a, b*) and divides to form the 2 cells of the teliospore (Fig. 1, F, *c, d*), while the stalk cell (Fig. 1, F, *e*) grows long and slender.

Soon after the last division the pair of nuclei in each cell of the teliospore fuse. At the time when the 2 nuclei become flattened against each other (Fig. 1, G) each contains a large spherical nucleolus and a number of delicate chromatin strands.

The nuclear membrane between the 2 now breaks down (Fig. 1, H) and the outline rounds out into an ellipsoid. The chromatin strands are fewer in number than the sum of those of the 2 component nuclei and are heavier. This may be due to a pairing of the strands; in fact, occasional hints of this can be seen, although in so minute a nucleus it would be difficult to prove. The nucleoles are still some distance apart. A slightly later stage is drawn in figure 1, I. The nucleus has grown, the chromatin strands are more uniform in diameter, and the nucleoles are fusing. In figure 1, J, the nucleolar fusion is complete. At no time has the nucleole been seen disconnected from the chromatin strands. It may be that here, as is maintained for higher plants, the nucleole occurs at a definite locus in a chromosome. If so, the nucleolar fusion here would be further evidence of chromosomal pairing at this stage. The figures G to J are drawn at the same magnification (×2,250). A comparison of the drawings shows that the volume of the nucleus has increased rapidly during the fusion.

The sequence of the stages represented in figure 1, K, L, and M, is not certain, but L and M probably represent later stages. Figure 1, M, resembles closely the synizesis of higher plants.

Figure 2, A, shows a teliospore (drawn at lower magnification than the preceding) at the time when nuclear fusion is in progress. In the lower cell the 2 nuclei have just combined; in the upper, fusion is complete. In figure 2, B, is drawn the mature teliospore with its firm wall, dense cytoplasm, and completely fused nuclei.

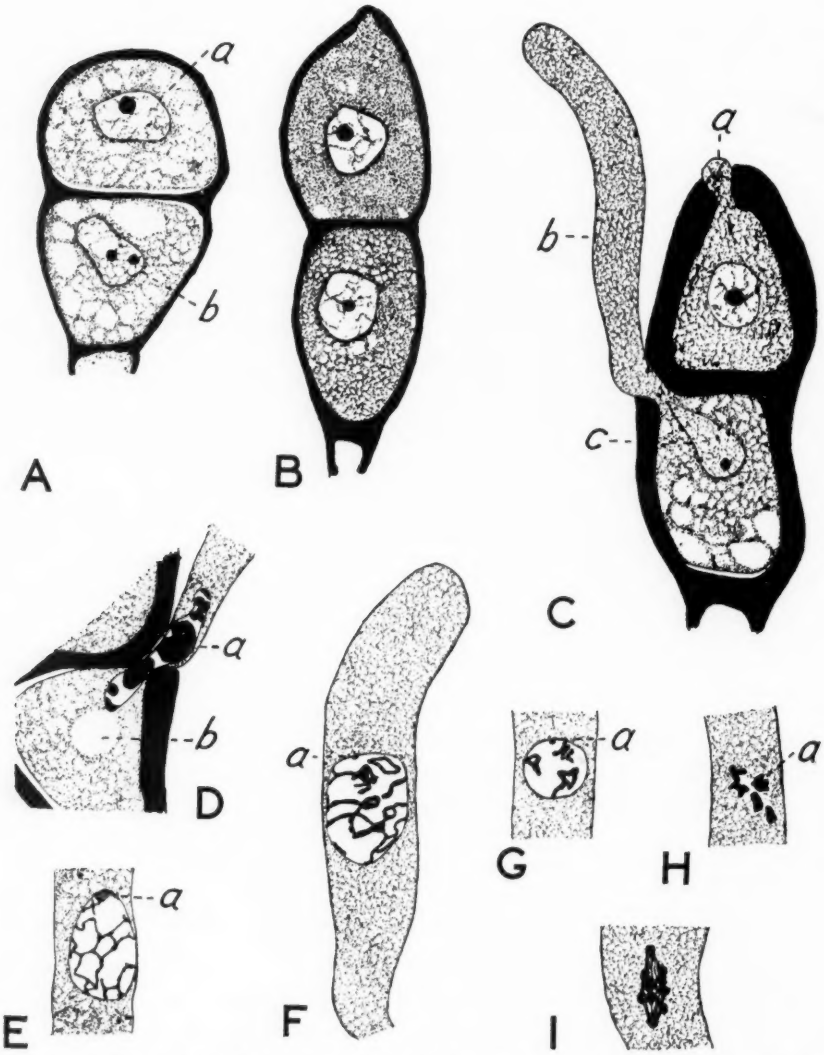

FIG. 2. *Puccinia malvacearum.* A, maturing, B, mature, and C, germinating teliospore; D, transit of nucleus to promycelium; E to H, prophases of heterotypic division; I, early anaphase. A to C, ×1,400; D to I, ×2,250.

After 2½ hours in a damp chamber the teliosori are covered with young promycelia. Figure 2, C, shows an early stage. The distal cell of the spore is just beginning to push out at *a* and the proximal cell has formed a hypha, *b*, as long as the spore. It contains only cytoplasm, however, for the nucleus, *c*, is still in the spore, although it is elongating in the direction of the germ pore.

The transit of the nucleus through the germ pore has been seen 2 or 3 times and in each case the condition in the nucleus is as represented in figure 2, D, *a* (drawn at high magnification). This concentration of the chromatin into lumps may be due, at least in part, to bad fixation, although it is not clear why this stage should be specially vulnerable when the stages just before and after it are well fixed. The exit of the nucleus leaves a vacuole, *b*, in the parent cell.

Immediately after its exit into the young promycelium the nucleus expands again (Fig. 2, E) and the chromatin loosens up into delicate strands. As the prophases of the first division progress, the chromatin threads shorten and thicken (Fig. 2, F) until finally the short, thick chromosomes can be recognized (Fig. 2, G). During this period a small granule (Fig. 2, E, *a;* F, *a;* G, *a*) appears at the distal end of the nucleus. It is probably not the nucleole. It may be a centrosome such as has been described for other fungi, but proof of this is lacking.

Next, the nuclear membrane disappears and the short, lumpy little chromosomes (Fig. 2, H) are scattered loose in the cytoplasm. Each consists of at least 2 parts and the one at figure 2, H, *a*, could easily be interpreted as consisting of 4, corresponding to the "tetrads" in the heterotypic division of higher plants. An attempt has been made in this and subsequent stages to determine the chromosome number. The minuteness of the division figures makes this difficult, but when the figures are clearest the number appears to be 5.

The spindle forms with its main axis parallel to the length of the hypha. Figure 2, I, shows an early anaphase with the dense little chromosomes crowded upon it. Figure 3, A, shows another anaphase with a broader spindle on which the chromosomes are better disposed for counting. There seem to be 5 in each group of daughter chromosomes. The little dark-stained spot at each pole again suggests a centrosome but, of course, may be only the denser meeting point of many fibers. Some 40 or 50 such division figures have been seen and, whatever it is, this dark-stained polar spot is regularly present.

In figure 3, B, is represented a typical late anaphase. The chromosomes have not moved back to the pole at an even rate: 1 pair, *a*, is still at the equator; another, *b*, *b*, is half way to the poles; and the rest, *c*, *c* (probably 3, if one may judge by the shape of the mass), are at the poles. The line

from pole to pole, composed presumably of fibers, is regularly present at this stage.

The second division follows quickly upon the first; in fact, a resting stage between the two is not readily found. Figure 3, C, shows the 2-cell promycelium, each cell with a nucleus in prophase. As these nuclei are even smaller than those of the first division, little detail can be made out.

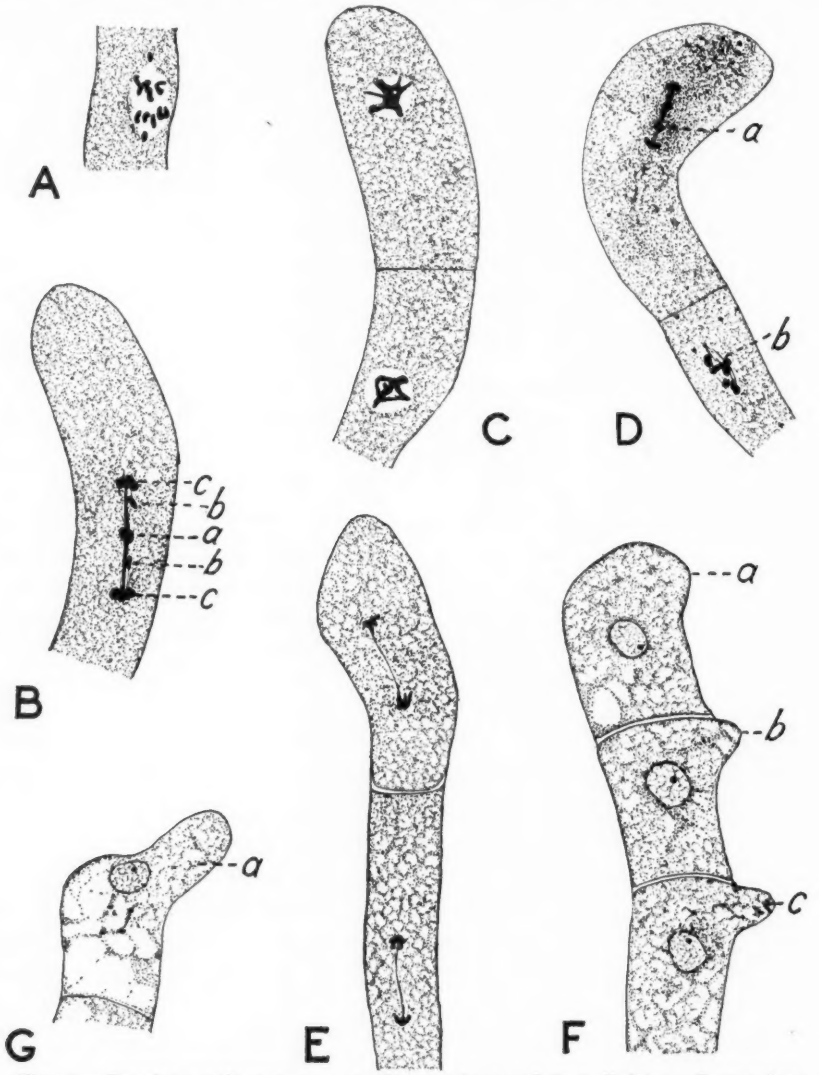

FIG. 3. *Puccinia malvacearum.* A, B, anaphases of first division; C, prophase, D, anaphases, and E, telophases of second division; F, G, beginning of sporidium formation. A to G, ×2,250.

In figure 3, D, the division has made unequal progress in the 2 cells; the lower cell at *b* is in early anaphase; in the upper, division is more advanced. In figure 3, E, are late telophases. The second nuclear division is quickly followed by cell division as the nuclei enter the resting condition.

The upper part of a 4-cell promycelium is shown in figure 3, F. Already, a blunt projection has pushed out at the apex of each cell (Fig. 3, F, *a*, *b*, *c*) to form the sporidia. Figure 3, G, shows the apical cell of a

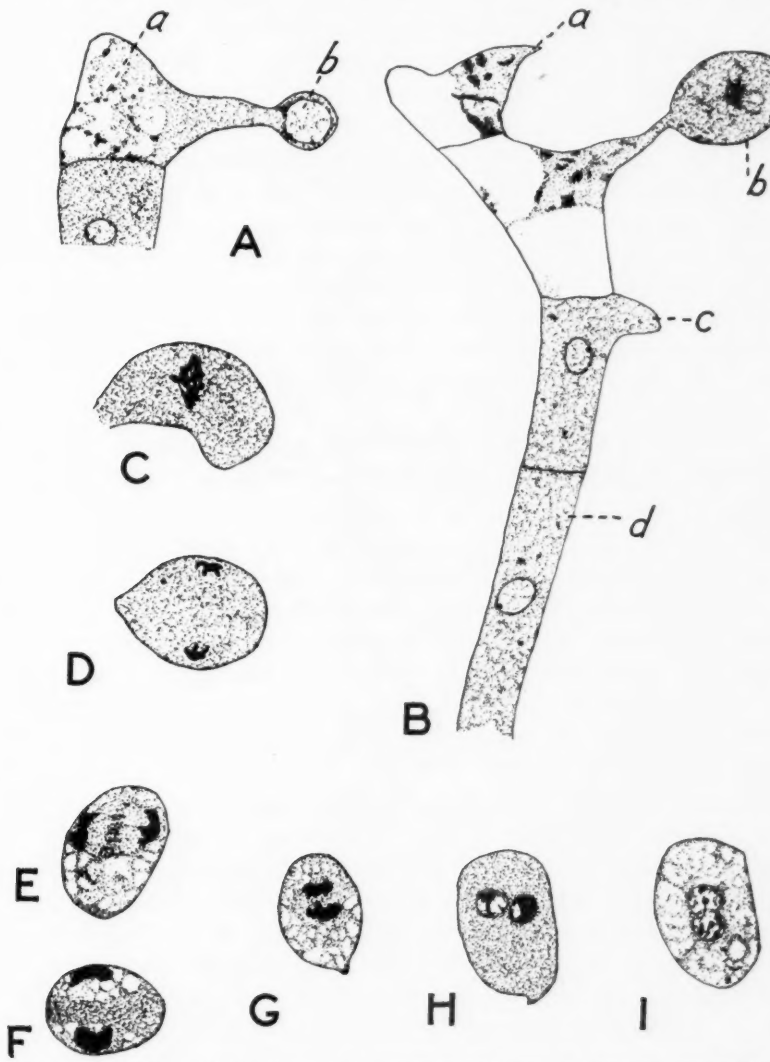

FIG. 4. *Puccinia malvacearum*. A, young sporidium; B, mature promycelium; C, metaphase and D to G, telophases of nuclear division in sporidium; H, I, mature binucleate sporidia. A to I, × 2,250.

promycelium somewhat further advanced in the process; a broad beak has formed at *a*.

As the sporidial beak elongates, a terminal swelling forms into which the nucleus moves (Fig. 4, A, *b*) leaving the parent cell, *a*, enucleate and with vacuolate cytoplasm.

Sporidia form in sequence on the promycelial cells, the apical cell commonly taking the lead. In figure 4, B, the terminal cell, *a*, has discharged its sporidium, the second cell, *b*, bears a nearly mature sporidium, the third, *c*, is just pushing out, and the fourth, *d*, shows no indication of spore formation.

The nucleus in the young sporidium in figure 4, A, *b*, is in the resting condition; that of the larger sporidium in figure 4, B, *b*, is in prophase of division. Figure 4, C, shows a metaphase of division with the spindle placed transversely in the sporidium. The chromosome number is probably 5. Ordinarily the sporidium is discharged before the division is completed. Later stages of division are abundant in newly discharged sporidia. Figure 4, D, E, F, and G, shows steps in the telophase and reconstruction of the daughter nuclei. Sometimes the plane of division is transverse, sometimes longitudinal. In figure 4, H and I, the division is complete and the binucleate sporidium is ready to germinate.

DISCUSSION

A number of cytologists have attempted to study nuclear divisions in the rusts, but the nuclei are so small that little detail can be made out with certainty. The earlier work (Rosen in 1892 (34), Poirault and Raciborski in 1895 (32), Sappin-Trouffy in 1896 (35, 36), and Juel in 1898 (20)) gave scant results. For some of the workers, even the formation of a spindle in connection with division was a debatable question. What could be seen was painstakingly recorded, but poor fixation had altered or destroyed many details. With later improvements in the technique, however, it has become recognized that nuclear divisions in the Uredineae are much like those of higher plants.

Statements concerning the chromosome number in rusts are usually hesitant and qualified; it is difficult to be certain. Poirault and Raciborski, in 1895 (32), found but 1 chromosome in a nucleus in *Peridermium pini acicolum* Link, *Puccinia liliacearum* Duby., *Aecidium leucospermum* D. C., and *Coleosporium euphrasiae* (Schum.) Wint. They state that "*. . . chaque noyau ne contient qu'un seul chromosome, mais c'est une caryokinése typique.*" Sappin-Trouffy in 1896 (35, 36), reviewed later by Tischler (38), studied representatives of the genera Endophyllum, Cronartium, Melampsora, Thecopsora, Gymnosporangium, Uromyces, Puccinia, Phragmidium, Triphragmidium, and Coleosporium and found the chromo-

some number to be uniformly 2. Juel, in 1898 (20), studied *Coleosporium campanulae* (Pers.) Lév. and found no differentiation of the chromatin into chromosomes during division. Maire, in 1900 (24) and in 1902 (25), found 2 chromosomes in divisions in *Puccinia liliacearum, P. bunii* D. C., and *Endophyllum sempervivi* Maire. Holden and Harper, in 1902 (18), found 6 to 10 chromosomes in *Colesporium sonchi-arvensis* Lév. Blackman, in 1904 (3), found "at least 10" chromosomes in the heterotypic division of *Gymnosporangium clavariaeforme* Rees but found that the vegetative divisions are "of such a simple nature that chromosome-formation is in complete abeyance." Christman, in 1905 (6), did not count the chromosomes but figured more than 2 in divisions in the aecium of *Caeoma speciosum* Fr. Olive, in 1908 (29), found 8 chromosomes in *Triphragmidium ulmariae* (Schum.) Link. Hoffman, in 1911 (17), studied the heterotypic division of *Endophyllum sempervivi* Lév. and found the chromosome number to be probably 8. Moreau, in 1913 and 1914 (26, 27, 28), found 2 chromosomes in *Phragmidium subcorticium* Schrank., *Coleosporium senecionis* Pers., and *C. sonchi* Pers. Colley, in 1918 (7), working on *Cronartium ribicola* Fischer, found more than 2 chromosomes and stated that "possibly 8 is the haploid number." Lindfors, in 1924 (22), found 4 chromosomes in nuclear divisions in *Puccinia arenariae* (Schum.) Wint. Dodge and Gaiser, in 1926 (10), studying *Caeoma nitens* Burrill, found the nucleus polarized in prophase and counted at least 14 loops.

The chromosome number for *Puccinia malvacearum* was not determined definitely, but the probable number is 5.

From the beginning of the work on nuclear divisions in rusts down to the present, workers have been divided on the question of the existence of a centrosome with or without polar radiations. Among the advocates of the centrosome are Juel (20), Holden and Harper (18), Blackman (3), Christman (6), Olive (29), Moreau (26), Colley (7), and Lindfors (22), but, here, as in all other questions affecting certain of the details of mitosis in the rusts, the minuteness of the nuclei and of the division figures makes it difficult to be certain.

There is a little evidence of a centrosome in *Puccinia malvacearum*. It is easy enough to find a small speck on the nuclear membrane of the resting nucleus and a tiny dark-stained area at the spindle poles during division, but this in itself does not prove the existence of a centrosome. The speck on the membrane may be merely one of the cytoplasmic food granules accidentally located there, and the dark spot at the spindle poles may be just the denser meeting point of many fibers. No polar radiations have been noted. Until the supposed centrosome can be traced through all stages of nuclear division and its behavior as a centrosome can be proved, it cannot be stated with certainty that a centrosome is present in *P. malvacearum*.

The one conspicuous object in resting nuclei of rusts is the single spherical nucleole. Students of rust cytology agree that during vegetative divisions this nucleole moves out into the cytoplasm, remains there through the metaphase, and gradually disappears during the anaphase and telophase. This has been observed and figured by Poirault and Raciborski (32), Sappin-Trouffy (36), Maire (24, 25), Christman (6), Olive (29), Dittschlag (9), Hoffman (17), Moreau (26, 28), and Fromme (14).

Quite different is the behavior of the nucleole during nuclear fusion. In *Puccinia malvacearum*, when the 2 nuclei fuse in each cell of the ripening teliospore, the 2 nucleoles, at first far apart, move closer together and then fuse. A similar nucleolar fusion in the teliospore (or in the aeciospore, in Endophyllum) has been observed in one or more rusts by Poirault and Raciborski (32), Sappin-Trouffy (36), Maire (25), Holden and Harper (18), Blackman (3), Hoffman (17), and Moreau (28). So far as can be judged, nucleolar fusion during nuclear fusion is of general occurrence in the rusts.

This nucleolar fusion is of interest in connection with the question of chromosomal pairing. Heitz (15) has shown that in a number of higher plants the nucleoles occupy definite loci on specific chromosomes. A nucleole appears first in the telophase as a ring around a thin place in a chromosome, then grows larger and rounds out. In the prophase of the next division the nucleole disappears gradually, again exposing at the same place the thin thread in the chromosome. The much-discussed "satellites" of chromosomes are merely the portions, large or small, of the chromosomes distal to the thin threads left at nucleolar loci. McClintock (23) and Burnham (5) have proved that in the reduction divisions of maize when the 2 "satellite chromosomes" pair the corresponding nucleoles are brought together.

It is perhaps a far cry from the nuclei of higher plants to those of rusts, but the regularity with which the nucleoles move together and fuse in the newly fused nucleus at once suggests that here, too, a nucleole occupies a definite locus in a chromosome and that it is the synaptic pairing of the homologous chromosomes that brings the nucleoles together.

It is an open question whether the synizesis in figure 1, M, is an artefact. If so, the chromatin at this stage is exceptionally sensitive to the fixing fluid, for figures such as this are not infrequent in otherwise well-fixed material. Moreover, such figures occur in other rusts and have been recorded by Holden and Harper (18), Blackman (3), Hoffmann (17), and Moreau (28).

The early stages of the prophase of the heterotypic division take place in the teliospore cell; the later stages wait until the nucleus has moved out into the promycelium. During transit, the nuclear content becomes so

greatly condensed that no detail of chromatin structure is visible and it is difficult to believe that the organization of the chromatin is maintained. This, too, has been observed in other rusts. Blackman (3) says that the nucleus is "very much constricted and condensed in its passage through the pore." Lindfors (22) speaks of the difficulty of making out the structure "*wegen des zusammengepressten Zustandes des Kerns.*" Although this transit takes place during division, the chromatin structure is not lost or impaired and division is resumed as soon as the nucleus rounds up again in the promycelial tube.

The 2 divisions follow in quick succession. If there is a true resting stage between the first and second divisions it is very brief. A few workers (3, 7, 18, 28) have figured it.

Binucleate sporidia appear to be fairly common in rusts. They have been observed by Poirault and Raciborski (32) in *Coleosporium euphrasiae;* by Sappin-Trouffy (36) in *Endophyllum euphorbiae sylvaticae* (D. C.) Wint. and *Puccinia malvacearum;* by Maire (24) in *Endophyllum sempervivi* Pers.; by Holden and Harper (18) in *Coleosporium sonchi-arvensis;* by Blackman (3) in *Phragmidium violaceum* Wint. and in *Gymnosporangium clavariaeforme* Rees; by Colley (7) in *Cronartium ribicola;* and by Lindfors (22) in *Puccinia arenariae.*

It has been usually taken for granted, as stated by Blackman (3, p. 329), that the nuclear division in the sporidium "appears to be entirely without significance and to be merely a precocious division of the nucleus in which the usual wall-formation is delayed" and that "the sporidia have been observed to give rise to a mycelium with single nuclei." A few (18, 32) have assumed that the binucleate sporidium grows directly into binucleate mycelium. In the case of *Puccinia malvacearum,* however, Ashworth (1) has figured binucleate sporidia and states that the early mycelium is uninucleate.

Lindfors (22) reports the unique case of *Puccinia arenariae* a leptopuccinia. When the teliospore germinates the nucleus divides twice but the promycelium remains bicellular, each binucleate cell of it giving rise to a single binucleate sporidium, which grows directly into binucleate mycelium.

Puccinia malvacearum is a rust without aecia or uredinia. It is maintained as a continuous series of telial generations unaccompanied, so far as known, by spermogonia. It is of interest, therefore, that there is no hint of degeneration in the reproductive processes. In fact, the reduction divisions here (in such details as can be seen in these tiny nuclei) appear to be as exact and efficient as the corresponding processes in higher plants.

SUMMARY

In *Puccinia malvacearum* teliospores are formed by binucleate basal cells each of which may by branching give rise to several spores in succession. Each spore initial divides into spore and stalk cell and the spore cell divides again to form the 2-cell spore.

The 2 nuclei in each cell of the spore fuse. The 2 large nucleoles in the fusion nucleus fuse and there is some evidence that the chromatin strands pair.

The long slender chromosomes, which wind about the nuclear cavity, now shorten and thicken into little lumps that are at least double and possibly quadruple. The nucleole and the nuclear membrane disappear. No sharply defined equatorial-plate stage has been noted; the chromosomes, probably 5 in number, lie scattered along the length of the spindle. The chromosomes divide and pass back to the poles, some arriving ahead of others.

A cell wall divides the promycelium into 2 cells and a second nuclear and cell division follows quickly.

Each cell pushes out a broad beak at its upper end, which swells at its tip to form the sporidium. The nucleus moves out into the young sporidium and divides, the later stages of the division taking place after the sporidium is discharged. Mature sporidia are regularly binucleate.

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A NEW SCLEROSPORA FROM NYASALAND

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(Accepted for publication August 25, 1932)

Collections within recent years have shown that we may expect the discovery of interesting new species of Sclerospora, such as *S. northi*[2] and *S. noblei*,[3] on wild grasses in regions in the Eastern Hemisphere where little intensive mycological work has been done. It was a fortunate circumstance, therefore, that Dr. E. J. Butler, Director of the Imperial Mycological Institute at Kew, was called to Nyasaland, Southeast Africa, to make a survey of the diseases of tea and tobacco, because during his years of constructive mycological work in India Dr. Butler had become thoroughly familiar with the Sclerosporas, the importance and destructiveness of which he had been one of the first to emphasize. While engaged in this survey he collected *S. graminicola* Sacc. Schroet. on *Pennisetum typhoideum* at the Makwapala Cotton Station near Zoniba, the first record of this species for that region, where it apparently causes considerable destruction, for Dr. Butler found as much as 30 to 40 per cent of the heads badly deformed and partly or completely sterile as a result of cumulative reinfection through continuous planting. Also in March, 1927, among the abundant clumps of wild grasses growing in Mr. Evans's tobacco estate at Bulaki, Nyasaland, Dr. Butler found a few tufts showing the shredding of the leaves into the tangles of fibers that are characteristic of infection by oogonial stages of Sclerospora. The host grass as determined at the Kew Herbarium proved to be *Eragrostis aspera* Nees., and material was brought back by Dr. Butler for further study.

On his return Dr. Butler and Mr. E. A. Mason, of the Bureau of Mycology, examined and compared these collections and found that the fungus on Eragrostis differed sharply from *Sclerospora graminicola* in that the resting spores were smaller and had bumpy protrusions from the oogonial wall. Knowing my interest in this genus, Dr. Butler generously gave me the material and hospitably extended to me the laboratory facilities of the Mycological Institute where part of this study was carried on in the summer of 1930. The fungus proves to be a strikingly distinct species that in the bumpy protruding wall and the small size of the resting spores and oospores is absolutely distinct from any species described hitherto. Also, it is known only on this host from this locality, and its parasitism of this species of grass adds another genus and species to the ever-growing list of gramineous

[1] Contribution No. 114, Laboratories of Cryptogamic Botany, Harvard University.

[2] Weston, W. H., Jr. A new Sclerospora from Fiji. Phytopath. **19**: 961–967. 1929.

[3] *Ibid.* A new Sclerospora from Australia. Phytopath. **19**: 1107–1115. 1929.

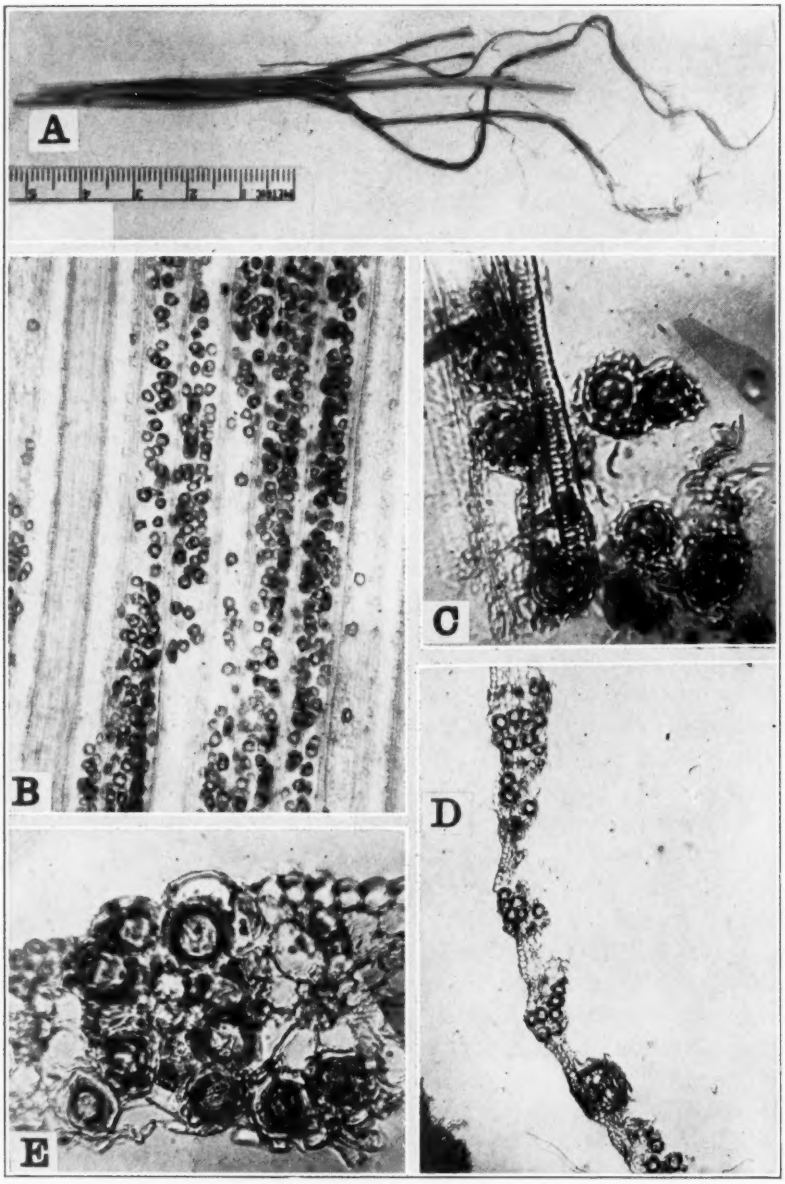

FIG. 1. A. Portions of leaves of *Eragrostis aspera* showing the disintegration of the leaf tissue into tangled fibers as a result of infection by *Sclerospora Butleri*. ×1. B. Surface view of a heavily infected portion of leaf tissue cleared in lacto-phenol to show the crowded masses of resting spores within. ×40. C. Bit of leaf tissue teased out to show the resting spores. ×175. D. Cross section of a slightly infected leaf showing the occurrence of the resting spores within. ×40. E. Similar cross section more highly magnified to show the position of the resting spores. ×175.

hosts for the Sclerosporas. In the hope that because of these features the fungus may prove of interest to others, a brief discussion of it is presented in the following note.

EFFECT ON THE HOST

On the Eragrostis the fungus produces a general withering and browning of the leaf until in later stages of development the mesophyll tissue between the bundles is destroyed (Fig. 1, B, D, E) and the leaf shreds out into tangled snarls of fiber. (Fig. 1, A.) This symptom is well known as characteristic of most of the species of Sclerospora in their oogonial phase. In this Nyasaland material the slender, elongate discolorations of the leaf tissue that appear early in the development of the parasite have almost disappeared and no traces of any conidial phase are present, nor did Dr. Butler encounter any in the field.

CHARACTERISTICS OF THE FUNGUS

Of the mycelium, as would be expected in an oogonial phase so far advanced in development, only fragmentary traces remain here and there.

The resting spores are developed in the mesophyll in great numbers, disorganizing the tissue until they come to occupy practically all of the space between the fibrovascular bundles. In leaves properly cleared (Fig. 1, B) they can be seen in this position before the disintegration of the epidermis of the leaf permits them to scatter. These resting spores, as is characteristic of the genus, comprise a heavy outer wall, which morphologically is the thickened modified wall of the oogonium, closely enclosing the single thick-walled oospore within. (Figs. 1, C, and 2, B.)

The oogonial wall is relatively thick, ranging from 4 to 10 μ (inclusive of protrusions), approximately $\frac{1}{5}$ of the total diameter of the resting spore. The surface is furnished with the blunt, rounded protrusions that are characteristic of the species and unknown for any other Sclerospora described hitherto. These protrusions, which may be rounded conical to tapering papillate or finger-like (Fig. 2), vary from 2 μ in width at the base and 2 μ high, up to 3 or 4 μ at the base and 4 or 5 μ high. They are solid, not hollow; pallid, almost hyaline, in marked contrast to the dark body of the oogonium wall; and distinctly modified in substance, reacting differently to treatment with stains or with chloro-iodide of zinc. The color of the main shell of the oogonial wall is variable in the many hundreds of spores examined but ranges from a pallid golden to a dark amber or resin, *i.e.*, from the Mars Yellow or Raw Sienna to the Sudan Brown or Antique Brown of Ridgway.[4]

[4] Ridgway, Robert. Color standards and color nomenclature. 43 pp.; 53 col. pls. Washington, D. C., 1912.

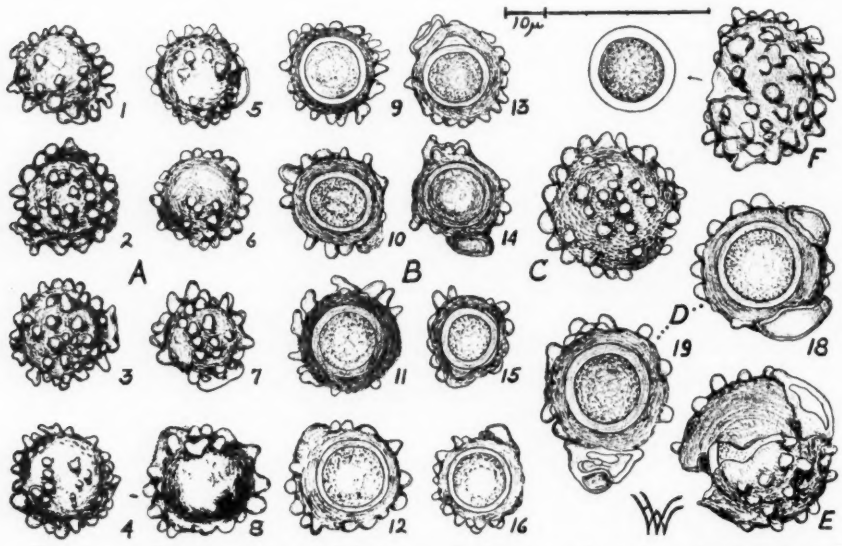

FIG. 2. A. Resting spores of *Sclerospora Butleri* in surface view, showing the pale, bullate, or papillate protrusions from the surface of the dark oogonial wall. ×500. B. Resting spores seen in optical section showing the hyaline protrusions, the dark body of the oogonial wall itself closely enveloping the spherical pallid oospore within. ×500. C. Surface view of a typical resting spore more highly magnified. ×700. D. Two resting spores seen in optical section showing, adhering to the exterior of the spore, structures that in 18 might be antheridia but, in 19, seem to be portions of the stomatal structure from the epidermis beneath which the spore was developed within the tissue of the leaf. ×700. E. Resting spore cracked open to show the relative size and position of the oospore within. ×700. F. Resting spore cracked open, freeing the enclosed oospore that now lies beside it. ×700. Camera-lucida drawings, scale in microns.

This outer oogonial wall fits closely to the single oospore within and enclasps it so adherently that only with difficult and careful manipulation can the oospore be freed. (Fig. 2, E and F). Rarely slight gaps are noticeable between the oospore wall and the inner surface of the enveloping oogonium coat that encloses it. (Fig. 2, B, 13, 15). The oospore is usually in an approximately centric position, as the oogonial wall is of almost even thickness throughout. (Fig. 2, B). Very seldom do any fragments of an oogonial stalk remain attached; a contrast to *Sclerospora noblei* in which this is a common occurrence. Very rarely also are adherent fragments seen that might be interpreted as traces of attached antheridia (Fig. 2, B, E); more commonly such fragments seem to be adherent remains of stomatal guard cells from the adjacent epidermis of the leaf. (Fig. 2, D).

In size the resting spores vary greatly, but, in general, the outer dimensions, including the protrusions, range from 29 to 40.9 μ, with average diameters of from 33 to 36.9 μ perhaps most commonly encountered (Table 1).

The single oospore within, almost exactly spherical, and the smallest of any Sclerospora yet known, ranges from 15 to 26.9 μ in diameter, most frequently 19 to 22.9 μ (cf. Table 1). The wall is smooth, hyaline, from 2 to 3 μ in thickness, most frequently averaging 2¼ to 2½ μ. The content shows a finely granular matrix, hyaline or of grayish protoplasmic color, with occasional oil globules that do not, however, make any definite pattern of associated droplets nor any definitely eccentric or centric position of single drops. Attempts to induce germination of the resting spores have all been unsuccessful thus far.

TABLE 1.—*Measurements of the diameters of 100 resting spores of Sclerospora Butleri, n. sp., and of 200 oospores, arranged in size classes to show frequencies*

Diameters of resting spores		Diameters of oospores	
Classes μ	No. of resting spores in 100	Classes μ	No. of oospores in 200
21 to 22.9	1	15 to 16.9	2
23 to 24.9	1	17 to 18.9	18
25 to 26.9	0	19 to 20.9	73
27 to 28.9	1	21 to 22.9	92
29 to 30.9	10	23 to 24.9	14
31 to 32.9	13	25 to 26.9	1
33 to 34.9	25		
35 to 36.9	24		
37 to 38.9	15		
39 to 40.9	9		
41 to 42.9	1		

IDENTITY OF THE FUNGUS

Even though the material is scanty and consists of resting spores alone, as no conidial phase is known, the fungus stands out as quite distinct from any of the Sclerosporas hitherto described. The small size of the oospores and the bullate or papillate character of the exospore are sharply distinguishing features. The size of the oospores, ranging from 15 to 26.9 μ and most commonly 19 to 22.9 μ, is distinctively smaller than that of the oospores of *Sclerospora noblei*, hitherto the smallest known, with their diameter most frequently 25 to 26.9 μ. Coupled with the small size of these oospores of this new Sclerospora on Eragrostis in Africa is the relative thickness of the wall, which commonly is 2 to 3 μ, approximating that of oospores in species of much greater diameter and twice as heavy as those of *S. noblei*, which commonly are 1 to 1.5 μ in thickness.

It is the bluntly rounded to papillate protrusions from the surface of the exospore, however, that most sharply distinguish this species. By these

protrusions the surface of the exospore is given an appearance wholly unlike the irregularly ridged, angularly winged, polygonally faced to patchily scabrous contour typical of most species. Nor does the bullate or papillate surface of the spores of this new Sclerospora approximate that of the spores of *Sclerospora noblei,* whose dark, bluntly rounded, knobby projections are of the same dark color as the remainder of the oogonial wall, few in number and sufficiently large and irregular in many cases to render the whole spore unsymmetrical. In this new species from Africa, on the contrary, the protrusions are pallid, in marked contrast to the dark body of the oogonium wall, numerous, often close together, and, although varying in size, in general follow the contour of the oogonium, so that the profile outline inclusive of the projections is usually approximately spherical. Not only do these structural characters mark this species as distinct, but also the host on which it occurs, *Eragrostis aspera,* is new to the long list of grasses parasitized by various species of Sclerospora.

For these reasons, even though the writer is reluctant to describe as new a fungus with an incompletely known life history, it seems necessary to recognize this as a separate species. It is therefore described as follows:

Sclerospora Butleri, n. sp. Oogonium (outer portion of the resting spore) approximately spherical to irregularly subspherical; 29 to 40.9 μ in diameter, most frequently 33 to 36.9 μ; pallid golden to dark amber or resin color (Mars Yellow or Raw Sienna to Sudan Brown or Antique Brown, Ridgway[5]), the dark body of the oogonial wall of relatively even thickness ranging from 4 to 10 μ exclusive of protrusions, the surface set with numerous bluntly rounded, papillate to finger-like protrusions, pallid, almost hyaline, in contrast to the dark body of the oogonial wall, and approximately 2 μ in width at the base by 2 μ high, to 3 or 4 μ at the base and 4 or 5 μ high. Oospores occupying an approximately central position within the enveloping oogonium, the oospores regularly spherical, small, most frequently from 19 to 22.9 μ in diameter, the wall hyaline, usually 2 to 3 μ in thickness, content with a finely granular matrix, hyaline or grayish, with one or several oil droplets not arranged in any definite pattern.

Germination of the resting spores not yet observed. Conidial or sporangial phase unknown.

Occurring on *Eragrostis aspera* Nees. at Bulaki, Nyasaland, Africa, collected by Dr. E. J. Butler in March, 1927.

Specimens deposited in the Farlow Herbarium, Harvard University.

Oogonia sphaerica vel irregulariter subsphaerica, 29–40.9 μ diam., saepissime 33–36.9 μ, flava vel flavo-brunnea ("Mars Yellow" vel "Raw Sienna" deinde "Sudan Brown" vel "Antique Brown," Ridgway); tunica

[5] See footnote 4.

colorata circumcirca aequali, 4–10 μ crassa protrusionibus exclusis; protrusionibus hyalinis vel pallidis, papillosis vel rotundato-conicis, 2–5 μ altis, ad bases 2–4 μ diam.; oosporis centricaliter dispositis, globosis, saepissime 19–22.9 μ diam., episporis hyalinis 2–3 μ crassis intus granulosis hyalinis vel griseis, guttulis 1–∝ irregulariter dispositis. Germinatione oosporae nondum visa. Conidiis vel sporangiis ignotis.

Hab. in foliis *Eragrotis aspera* Nees. Bulaki, Nyasaland, Africa, coll. E. J. Butler, Mar. 1927.

DISCUSSION

Our knowledge of this Sclerospora, even though at present rather scanty, involves certain interesting features.

In the first place, the occurrence of this Sclerospora on *Eragrostis aspera* adds another representative to the list of gramineous hosts known to harbor species of this destructive genus. Of the distribution of the fungus on this host little can be said, for, while it is known at present only from this one locality in Bulaki, Nyasaland, the host on which it occurs, as indicated by the collections in the Kew Herbarium and by the ranges of geographical distribution given in various floras, is common through tropical Africa, occurring from the seacoast up to over 6,000 ft., from Natal and the Cape, up through the Belgian Congo, Tanganyika, Kenya, the British East Africa Protectorate, Zambesia, Sudan, Uganda, Belgian Congo, Nigeria, Rhodesia, Angola, Sierra Leone, Kamerun, the Gold Coast, up into Somaliland, Abyssinia, and Eritrea. It seems wholly probable, therefore, that more intensive mycologic collecting in these regions may discover this species of Sclerospora elsewhere throughout the range of its host. It is even possible it might be found as well on other related hosts in this region or elsewhere in Nyasaland, from which as yet only this fungus and the wide-spread *Sclerospora graminicola* collected by Dr. Butler on *Pennisetum typhoideum* have been reported.

In the second place, the fungus is of interest in itself because of the distinctive pallid, papillate protrusions from the surface of the exospore, a structural feature quite in contrast to the contour and surface sculpturings of any species of Sclerospora known hitherto. So unusual is this wall character for a member of this genus that it might be argued that this fungus may not prove to be a Sclerospora at all. The small size of the entire resting spore and of the oospore contained within, the papillate or bullate protrusions from the exospore surface, the lack of any recognizable extensive peronosporaceous mycelium, and the lack of any conidial or zoosporangial nonsexual reproduction typical of the downy mildews, might be advanced as evidence that this is one of the Chytridiales allied perhaps to Cladochytrium or Physoderma. Yet, the size, although smaller than that

of any other known Sclerospora, is approached by that of *Sclerospora noblei*, while the bullate surface of the exospore, although a character never encountered hitherto in any species of Sclerospora, is not in itself a valid reason against inclusion in this genus, since, in species of other genera of the Peronosporales, such as Albugo, strikingly different surface sculpturings may occur. The lack of any conidial phase in the present scanty material may mean no more than that such a phase has not yet been found, for, until recently, no such stage had been discovered for *S. noblei*, while in the case of *S. farlowii* and *S. northi* the justice of their inclusion in Sclerospora is unquestioned, even though no conidial phase has yet been discovered for either of them. Also, the absence of a recognizable peronosporaceous mycelium is not unusual in mature oogonial material of most species of Sclerospora when leaves are in an advanced stage of shredding, as in this. Moreover, the fact that this fungus parasitizes one of the Gramineae and produces ultimately the shredding of the leaves into tangled fibers is an indication of its being one of the Sclerosporas, a conclusion supported still further by the fact that the resting spore has the general Sclerospora structure of a heavy exospore closely enveloping the single oospore within. Obviously, there is great need for further study of the life history of this species, and it is to be hoped that some one strategically located in the region where it occurs will carry on investigations to increase our scanty knowledge of the fungus and to settle any doubts as to its identity and relationship.

As to the possible economic importance of this fungus, little can be said at the present state of our knowledge concerning it. It might, of course, have the beneficial effect of helping to keep down this grass in its rôle as a troublesome weed in coffee plantations and other cultivated areas. On the other hand, it should be noted that from the Gold Coast *Eragrostis aspera* is reported to be a forage grass of value, being relished by horses at all times, although eaten by cattle only when it is young, while in Rhodesia large quantities are grown and sold in the market for hay and the racing stables use it commonly. If this Sclerospora proves as seriously destructive to this grass as other Sclerosporas are to many other valuable cultivated or forage Gramineae, it may well become an important disease. Moreover, the possibility of its transferring to other more valuable grasses cannot be overlooked. The genus Eragrostis, occurring in the tribe Festuceae of Hitchcock under the subfamily of Poacoideae, comprises over a hundred species, both tropical and temperate, some of which are of importance, while among the 30 to 40 genera in the same tribe there are others, such as Bromus, Phragmites, and Arundo, already known to be hosts for species of Sclerospora.

SUMMARY

This paper discusses a Sclerospora causing browning, withering, and ultimate shredding of the leaves of *Eragrostis aspera* in Nyasaland, where it was collected by Dr. E. J. Butler.

In its resting-spore stage, the only reproductive phase yet known, the surface shows distinctive, rounded, bullate or papillate protrusions from the darker exospore, while the oospores within are the smallest of any Sclerospora yet encountered, even smaller than those of *Sclerospora noblei.* The species is described as *S. Butleri,* n. sp., and its distinguishing features of structure and surface configuration are discussed.

That the host, *E. aspera,* is an addition to the list of grasses parasitized by species of Sclerospora is noted, and questions of distribution and possible economic importance are discussed.

LABORATORIES OF CRYPTOGAMIC BOTANY,
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STEM CANKER OF CROTALARIA SPECTABILIS CAUSED BY DIAPORTHE CROTALARIAE, N. SP.

GEORGE F. WEBER

(Accepted for publication August 19, 1932)

INTRODUCTION

In the summer of 1930, at Gainesville, Florida, *Crotalaria spectabilis* Roth. plants were found bearing cankered areas on the stems which apparently had been caused by a fungus. Cultures of a fungus were obtained from the pith under the cankered areas and were observed for a year, but no signs of sporulation developed. During 1931, additional isolations were made and the fungus obtained was indistinguishable from that obtained during the previous season. Blossoming Crotalaria plants, grown in pots in the greenhouse, were inoculated with mycelium of the fungus and in 10 days stem lesions developed that, after 2 weeks, were typical of those on the plants in the field. Pycnidia developed on the inoculated plants, which contained spores typical of the genus Phomopsis. Careful observations in the field revealed similar fruiting structures on diseased plants and buried perithecia with long hair-like necks containing Diaporthe-like spores on old Crotalaria stems of the previous season's growth. The pathogenicity of the organism has been established, its imperfect and perfect stages have been connected in culture and through inoculation experiments, and the disease and the parasite are described herewith.

THE DISEASE

While studying Crotalaria diseases (3), in 1930, stem canker was discovered in a small planting of *C. spectabilis* at Gainesville. During the following season it reappeared in a more epidemic form, and further observations showed that it was present in all plantings of *C. spectabilis* in the vicinity of Gainesville. It was also found in the vicinity of Leesburg and Lake Alfred. The percentage of infected plants at Leesburg and Lake Alfred was about the same and relatively small, but at the station where Crotalaria had been planted on the same land during consecutive years and the old stems from previous seasons were used as a mulch for trees bordering on the field the percentage was very high. In this planting more than 90 per cent of the plants were infected and 30 per cent were killed by blossoming time. Other fields visited in 1931 showed from 15 to 20 per cent infected plants. The disease has been found only on *C. spectabilis*, at present the most extensively grown species in the State.

Description. The disease appears first in summer when the host plant is in bloom. At this time the plants are from 3 to 6 ft. high and the stems are from $\frac{1}{2}$ to $1\frac{1}{2}$ in. in diameter at the soil line.

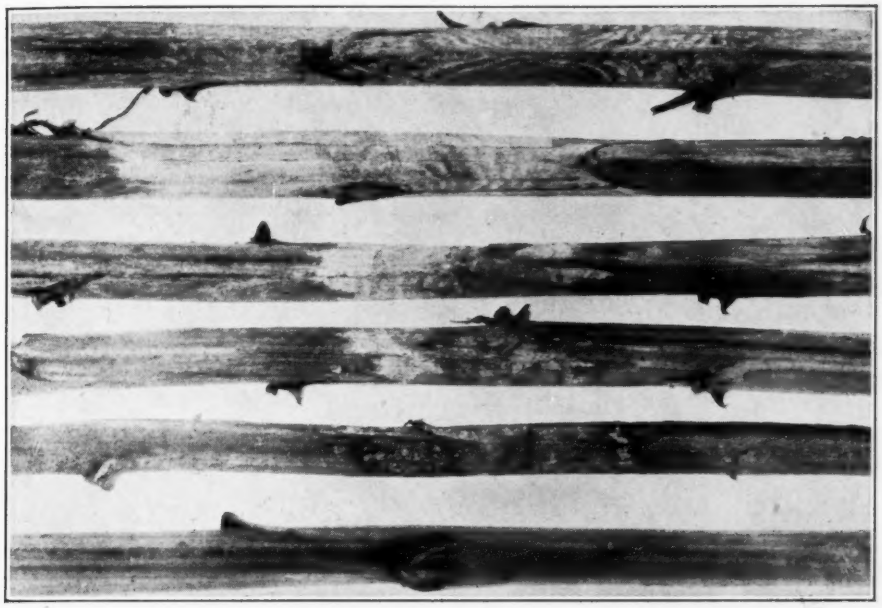

FIG. 1. Stem cankers on Crotalaria caused by *Diaporthe crotalariae*, showing various stages of development.

The stem lesions (Figs. 1 and 2, A) usually occur a foot or more above the soil line and below the branches of the inflorescence on plants in thick stands, while they occur at the soil line or within a foot of it in the more bushy plants of thin stands.

With few exceptions infection of the main stem occurs through leaf or branch scars. Having entered the stem, the fungus invades all parts of it rapidly, involving an area of 6 to 8 in. on each side of the infection point in a few days. As a rule, the stems are invaded for a distance of several inches before the parts of the plant above show symptoms of decline. The young lesions are a wood-brown (4) color, at first, but, as they enlarge, they become zonated by the formation of grayish white bands of mycelium underneath the epidermis (Fig. 1). This type of zoning is very characteristic of the disease and becomes more pronounced until the plants are killed. On plants growing in thin stands that develop branches close to the ground, infection usually takes place around the base of the branches. Lesions on such plants are less extensive and less characteristically zoned, although infected plants usually are killed.

With few exceptions pycnidia develop sparingly on the lesions. (Fig. 3, A). Occasionally, a lesion was found thickly dotted with imbedded pycnidia, which became erumpent during wet weather, and conidia exuded

FIG. 2. A. *Crotalaria spectabilis* plants, showing the disease as whitish areas on the lower portion of a number of the stems. B. One-year-old Crotalaria stems used as mulch. On these the perithecial stage was found.

from them in yellowish tan tendrils or masses. Conidia were seldom found, however, until after the plant was killed.

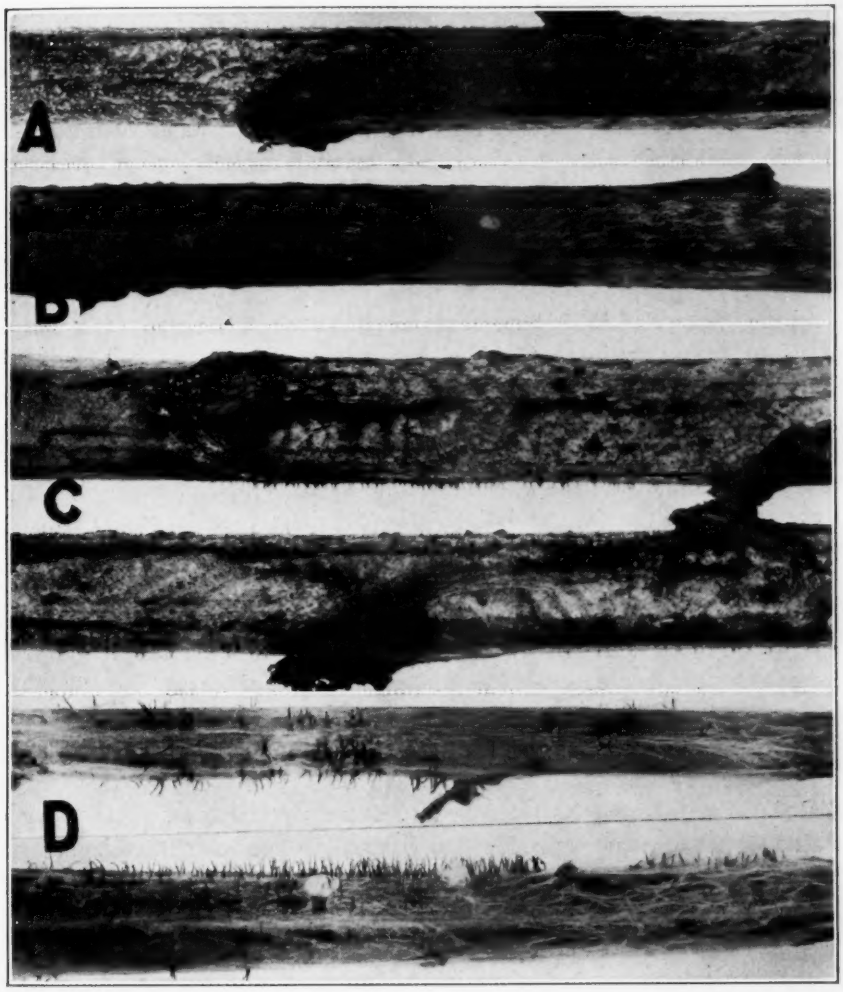

FIG. 3. Diseased Crotalaria stems. A. Pycnidia with spore horns exuding. B. Mycelium. C. Perithecia from nature. D. Perithecia developed in culture.

The perithecial stage of the fungus was found on current season's growth several months after it had been killed and on old stems of the previous season's crop. (Fig. 3, C.)

THE CAUSAL ORGANISM

Isolations. The first isolations were made from cankers on the stems. Considerable difficulty was experienced because of the loose porous pith,

in disinfecting the diseased material sufficiently to eliminate contaminants. Successful isolations were obtained, however, when the ends of the diseased stalks were recut in the laboratory and immediately sealed with melted paraffin, previous to disinfection in corrosive sublimate 1:1,000 for 15 minutes. The organism secured grew well on potato-dextrose agar, producing a dirty-white, flocculent, aerial growth characteristic of species of Phomopsis. A large number of isolations were made from these diseased areas in which the same fungus was obtained consistently. Cultures of this organism have been kept on potato-dextrose agar and transferred in the laboratory for a year and a half and no signs of sporulation have developed. On sterilized Crotalaria stems it produced pycnidia and pycnospores sparingly.

In the summer of 1931 spore horns were observed exuding from pycnidia on lesions on plants in the field. These specimens were examined in the laboratory and were found to contain both the small ovoid spores and stylospores characteristic of the genus Phomopsis. Cultures obtained from the infected pith underneath stem lesions and from single pycnospores obtained from the exuded spore horns were indistinguishable and resembled in every respect the fungus isolated the previous year from the diseased areas on the plant.

After the disease was definitely found to be caused by a species of Phomopsis, a search was made on old diseased plants for a Diaporthe that might possibly be the perfect stage of the fungus. Several piles of one-year-old Crotalaria stems, used as mulch around fruit trees, showed that a good percentage of the stems bore perithecia of a Diaporthe. (Fig. 2, B.) Single ascospore isolations were made and the resulting mycelial growth on potato-dextrose agar was comparable to that produced by the fungus isolated from infected host tissue and from pycnospores. The cultures obtained from ascospores produced perithecia on potato-dextrose agar after 3 weeks, which were comparable with those on old Crotalaria stems. The mature asci and ascospores thus produced in culture were indistinguishable from those developed in nature on Crotalaria stems. Sterilized Crotalaria stems were inoculated with potato-dextrose-agar cultures obtained from mycelium in the host tissue, from pycnospores, and from ascospores. After several weeks the stems inoculated with mycelium originating from ascospores showed perithecia typical of Diaporthe and similar to those found in nature on old Crotalaria stems. (Fig. 3, D.) Careful examination of these cultures a week later showed that all of them were producing both pycnidia and perithecia containing mature pycnospores and ascospores, respectively. The stems inoculated with cultures obtained from isolated mycelium and pycnospores produced pycnidia but not perithecia.

Pathogenicity. Blossoming Crotaliaria plants, growing in greenhouse pots, were inoculated with mycelium, pycnospores, and ascospores of the fungus. The mycelium growth on the surface of agar plates was cut in strips for convenience in inoculating plants. The spores were used in water suspensions. The inoculum in all instances was placed on the leaf scars where leaves were freshly removed or in incisions made through axillary buds. The inoculated areas were then closely covered with adhesive tape, which extended about an inch above and below the wound. The disease began to show on the stems beyond the tape-covered areas after 14 days. The data obtained from the experiments are shown in table 1.

TABLE 1.—*Results of inoculations on Crotalaria spectabilis with Diaporthe crotalariae, December 1931*

Source of inoculum	Number of plants	
	Inoculated	Diseased
Mycelium	18	18
Pycnospores	12	10
Ascospores (nature)	14	9
Ascospores (culture)	15	10
Sterile water	6	0

These inoculation experiments show that the mycelium and spore forms of the fungus are pathogenic on *Crotalaria spectabilis.* Characteristics of the disease produced by each of the inoculations were similar, and, after 3 or 4 weeks, they could not be distinguished, except by the labels. The plants inoculated with mycelium developed a higher percentage of infection and the resulting disease developed more quickly than that present in plants inoculated with ascospores or pycnospores. Four weeks after inoculation pycnidia appeared on several of the plants inoculated with pycnospores and ascospores. The diseased portions of these stems were removed and placed in moist chambers in the laboratory and after several weeks perithecia developed on all of them. The perithecia were imbedded between the wood and bark and had long hair-like beaks comparable to those found in nature and those developed on agar and sterilized Crotalaria stems. Both spore forms that developed on the inoculated plants were comparable with those found in nature.

Thus, briefly summarizing, the pycnidia and pycnospores found in nature were comparable with those produced in culture from spore isolations. The ascigerous stage developing on the host from pycnospore inoculations was the same as that found in nature. Likewise, the perithecia and ascospores developing from isolated ascospores on the host and in culture were

the same as those found in nature. The ascigerous stage was produced from the imperfect stage through the host and the imperfect stage from the ascigerous stage. The ascigerous stage was not developed in artificial culture from the isolated mycelium or pycnospores.

Taxonomy. A careful study of the literature failed to reveal a similar fungus on any species of Crotalaria. However, it was learned that Diaporthe has been reported on plants in closely related genera, but these are apparently quite different from the species discussed herein. Other diseases of economic importance caused by similar fungi have been reported by Harter and Fields (1), Jenkins (2), and Wolf (5) on sweet potato, rose, and citrus, respectively. Since *D. citri* (Faw.) Wolf is very common in most citrus groves and *C. spectabilis* is grown extensively as a cover crop in these plantings, some connection might be possible, but cross inoculations with the citrus organism failed to produce infection on the crotalaria.

It has been concluded after careful study that the disease on Crotalaria is undescribed and is caused by a hitherto undescribed fungus. Consequently, the parasitic fungus is described as follows:

Diaporthe crotalariae, sp. nov.

Synonym: Pycnidia—**Phomopsis crotalariae,** n. f. nom.

Pycnidial stage: Pycnidia on killed areas of stems imbedded under epidermis, irregular to subglobose, scattered, black, thick-wall, simple or chambered, pseudoparenchymatous, rupturing irregularly, erumpent at maturity, 200–450 μ in diameter. Pycnospores: ovate to elongate, hyaline, guttulate, unicellular, 5.9–10 × 1.9–2.8 μ, average 7.73 × 2.88 μ. Sporophores: simple hyaline tapering to apex, 15.4–26.6 μ long. Stylospores: not common, slender, hyaline, hooked, 16.6–30.94 × 1.8–2.3 μ.

Perithecial stage: On dead stems of the host 3 months to 1 year after being killed, single or gregarious, imbedded in stromata between bark and wood, globose to lens-shape, black, pseudoparenchymatous, walls 220–380 μ in diameter. Beaks protruding through bark, 1–2 mm. long, mostly 1–1¼ mm. tapering, black with brownish rounded tip, hairy on surface, ostiole about ¼ diameter of beak, apical pore circular. Asci: subsessile, elongate-clavate, sides often irregular, apex slightly thickened with pore, average 34.01 × 6.85 μ, 44 per cent measure 34.2 × 6.84 μ, aparaphysate. Ascospores: irregularly biseriate, hyaline, ellipsoid, obtuse, bicellular, slightly constricted at septum, each cell biguttulate at maturity, 9.12–12.31 × 2.28–3 μ, average 10.95 × 2.5 μ. (Fig. 4.)

Produces stem and branch cankers on *Crotalaria spectabilis.* Pycnospores found on recently killed parts, while ascigerous stage found on same parts after 3 to 6 months. Type material in the herbarium of the Florida Agricultural Experiment Station, Gainesville, Florida.

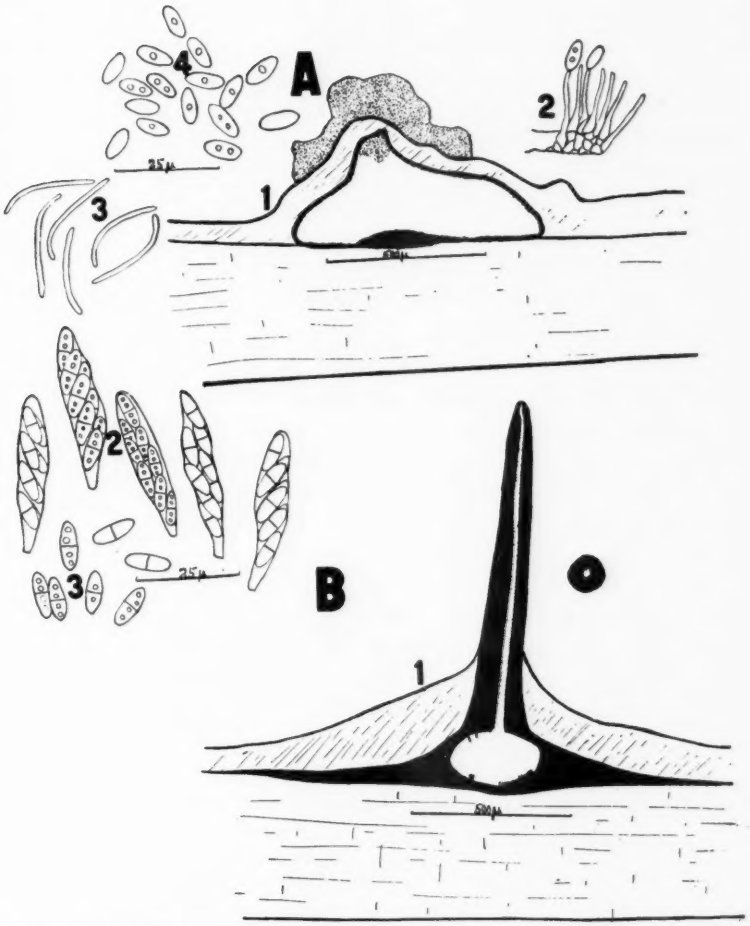

FIG. 4. Semi-diagramatic camera-lucida drawings of spore forms of *Diaporthe crotalariae*. A. (1) Pycnidium and exuded pycnospores; (2) sporophores; (3) stylospores; (4) pycnospores. B. (1) Longitudinal section of perithecium and cross-section of beak; (2) asci; (3) ascospores.

SUMMARY

A stem disease of *Crotalaria spectabilis*, distributed in central Florida, is described for the first time.

In certain fields at blooming time 30 per cent of the plants have been killed and 90 per cent infected by the disease.

The disease has been reproduced in inoculation experiments by using isolated mycelium, pycnospores, and ascospores of the fungus as inoculum.

The ascigerous stage has been obtained from the imperfect stage through inoculated plants. The imperfect and ascigerous stages have been obtained both in culture and through inoculated plants from the ascigerous stage.

The imperfect stage of the fungus is described as *Phomopsis crotalariae*, n. f. nom., and the ascigerous stage is described as *Diaporthe crotalariae*, sp. nov.

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THE PATHOLOGY OF TOBACCO BLACK SHANK

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(Accepted for publication August 15, 1932)

INTRODUCTION

Nearly every study that has been made of the tobacco disease, black shank, caused by *Phytophthora nicotianae* Breda de Haan, has made reference to the rapidity with which infected plants wilt and die. In most cases only 4 or 5 days are required to produce a wilting that involves the entire plant, and, after 2 or 3 more days, the plants succumb. There appears to be too little destruction of the cortex and pith to result in rapid collapse and subsequent death of the plant, especially since the xylem tissues are intact or only slightly invaded. Nolla (15), in his account of the histology of the disease as it occurs in Puerto Rico, states that the hyphae, which are both intra- and intercellular, invade parenchyma tissues and cause their dissolution.

Up to the present, the pathological studies of this disease, both as it occurs in the East Indies and in this country, do not account for the rapidity with which infected plants wilt. It seemed advisable, therefore, to inquire into the possibilities of the production of toxins, enzymes, acids, or alkalis to explain this phenomenon. The literature on these subjects is too voluminous to be summarized herein, but the following investigations are deemed pertinent to the present study.

Although the matter of toxin production by plant pathogens is not yet completely understood, it has received considerable attention. As long ago as 1913, Hutchinson (13) performed experiments with *Bacterium solanacearum* E. F. Sm., the organism causing tobacco wilt, which led him to believe that the production of toxic substances is, in the last analysis, responsible for the symptoms exhibited by diseased plants. Coons (5), in 1916, concluded that the wilting of potatoes caused by *Fusarium oxysporum* Schl. is attributable to poisons generated by this fungus. Additional evidence adduced by Haskell (9), in 1919, discredited the previously accepted theory advanced by Smith (19) 20 years before, which held that plants infected with species of Fusarium wilt because the mycelia in the xylem block the tracheae, thus shutting off the supply of water to the leaves. Of this, Haskell says, "The writer has examined the stems of many plants microscopically and found no instance of a stoppage of the trachea sufficient to shut off the passage of sap. . . . The theory that one or more toxines are responsible is much more plausible." Young and Bennett (23), work-

[1] The writer wishes to express his appreciation to Dr. F. A. Wolf, at whose suggestion the present problem was undertaken and with whose help and guidance it was completed.

ing with the same disease, definitely established the fact that *F. oxysporum* produces a substance toxic to potato plants. Gross (8) found that the killing of potato plants by *F. eumartii* C. W. Carpenter is not due to a blocking of the tracheae.

In 1924, Farrell (7) reported that the wilting of tomatoes in Kansas by *Fusarium lycopersici* Sacc. is caused by an enzyme. White (22) and Haymaker (10), however, working with the same disease, concluded that this wilting is the result of the production of toxic substances. Bewley (3) reported that the so-called "sleepy disease," another tomato wilt, is due to an exoenzyme secreted by *Verticillium albo-atrum* Reinke and Berth. Picado (17) subsequently showed that the symptoms of sleepy disease are caused by a chronic poisoning of the plant tissues.

Ajrekar and Bal (1) suspected that toxins are produced by the cotton-wilt organism, *Fusarium vasinfectum* Atk. In 1926 Rosen (18) demonstrated that 2 separate and distinct toxins, one an alkaline substance and the other a salt in the form of a nitrite, are produced by the cotton-wilt pathogen. Brandes (4) stated that toxins are probably a proximate cause of banana wilt, *F. cubense* E. F. Sm. The green mold, *Penicillium expansum* Link, was described by Barnum (2) as being capable of secreting substances toxic to plants. Fahmy (6) found that *F. solani* Schl. excretes a toxic material that "produces a rapid wilting of cut bean stems when these are placed in the filtrate of the fungus." The halos surrounding lesions produced by tobacco wildfire, *Bacterium tabacum* Wolf and Foster, have been attributed by Johnson and Murwin (14) to a toxin.

In his experiments with *Fusarium solani,* Fahmy (6) noted the increase in the degree of alkalinity accompanying a corresponding increase in the age of the culture, but he does not regard this alkalinity as being responsible for wilting. In the case of stem rots caused by *Sclerotium rolfsii* Sacc., Higgins (11) states that the death of cells subjacent to those invaded by the fungus is due to the toxic action of oxalic acid secreted by the fungous hyphae. His paper contains a summary of investigations concerning the production of oxalic acid by various bacteria and fungi. Smith (20) concluded that the pathogenicity of *Botrytis cinerea* Pers. to lettuce plants is due to the formation of oxalic acid, while Peltier (16) expressed the opinion that the toxic substance is an organic acid other than oxalic.

The results of an inquiry into the production of toxins, enzymes, and acids by *Phytophthora nicotianae* are presented as an explanation of the rapid wilting of tobacco by this pathogen.

TOXIN PRODUCTION IN CULTURE

Potato broth was found to be a favorable substrate for a rapid mycelial growth by *Phytophthora nicotianae.* A very satisfactory growth also was

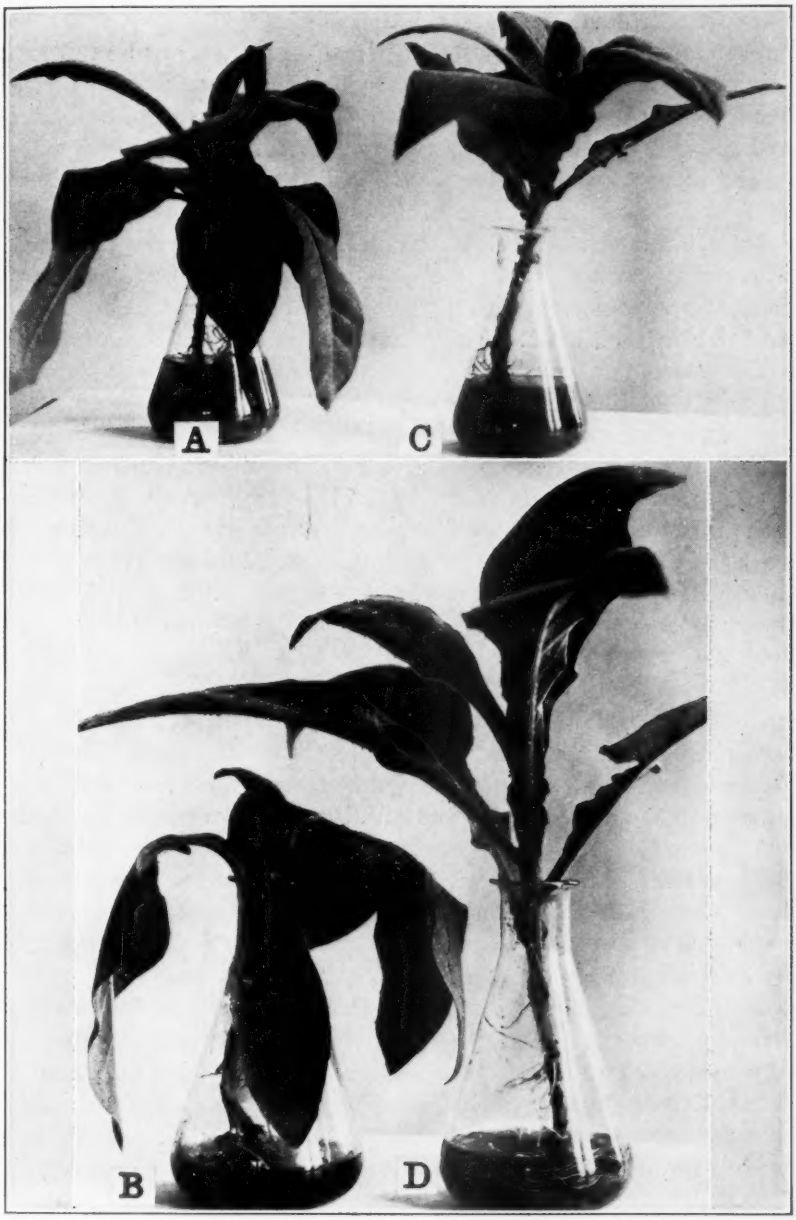

FIG. 1. A and B. Wilting of tobacco plants. A. In filtrate from 13-day-old potato-broth culture of *Phytophthora nicotianae*. B. In filtrate of material from infected tobacco plants. C. andD. Controls. C. In noninoculated potato broth. D. In filtrate from material from healthy tobacco plant. A and C photographed after 1 hour and B and D after 1½ hours.

obtained with flasks of Richards' solution diluted by ½ with distilled water. Toxin production was first tested after the organism had grown for 9 days in flasks containing potato broth. The broth was filtered through a fine grade of filter paper on a Büchner funnel. Microscopic examination showed the absence of the organism from the filtrate. A healthy tobacco plant was uprooted, washed free of soil, and its roots were immersed in this filtrate. A similar plant, placed in noninoculated potato broth adjusted to the same pH concentration, served as a check. In 45 minutes the plant in the filtrate was wilted, while the control remained normal. When this experiment was repeated with potato broth in which the pathogen had grown for 13 days, a period of 30 minutes sufficed to cause marked wilting, while the control remained turgid. Figure 1, A and C, shows these 2 plants an hour after the beginning of the experiment.

The same procedure was used to test the presence of toxins in cultures grown in Richards' solution. In 17-day-old cultures, 30 minutes sufficed to produce wilting. Plants placed in noninoculated Richards' solution as controls remained turgid. Because of the rapidity with which plants wilted when placed in filtrates from inoculated potato broth and Richards' solution, it seems probable that wilting was accomplished by toxic materials produced by the organism.

TOXIN IN DISEASED PLANTS

To obtain additional evidence of the production of toxin by *Phytophthora nicotianae,* infected tobacco plants that had been grown in the greenhouse were used. The diseased portion of the stems was removed and cut into pieces to facilitate maceration with a mortar and pestle. Sand that had received washings with water and sulphuric acid was used in the mortar as the abrasive agent. After grinding, a small quantity of distilled water was added to the macerated material, which then was stored at 34° F. for 18 hours. Extraction for only a few minutes sufficed to cause marked wilting.

The material was then filtered through a Büchner funnel, and a healthy tobacco plant was placed in the filtrate. A similarly prepared filtrate from healthy tobacco stems served as a control. After 45 minutes, the plant in the filtrate from diseased stems was badly wilted, while the control remained normal. These plants are shown in figure 1, B and D, as they appeared after 1½ hours.

This experiment was repeated with infected field-grown plants. In this case 1 hour was required to wilt the plant whose roots were immersed in the filtrate from black-shank lesions, while the control remained turgid.

Hursh (12) has pointed out that too much emphasis is being placed on the wilting of plants following their immersion in filtrates of various fungi,

since in many cases the wilted plants will regain their normal appearance on being removed from the filtrate and placed in fresh water. With this in mind, wilted plants were removed from the filtrates of cultures of *Phytophthora nicotianae* and placed in fresh water, but, when badly wilted, they did not recover. Recovery seems to be conditioned by the length of time plants are exposed to the toxin and by the degree of collapse.

NATURE OF THE TOXIN

Macerated tissues from diseased plants were allowed to stand in distilled water for 18 hours at a temperature of 34° F. Crystals of $MgSO_4$, which may be used to precipitate proteins, were added to the liquid obtained by filtration of this material. As the concentration of $MgSO_4$ approximated saturation, perceptible floccules were formed. When this material was again filtered, the floccules were retained on the filter paper. After several washings with water to remove any $MgSO_4$, this filter paper, along with the precipitate it contained, was placed in a flask of distilled water.

A healthy tobacco plant with roots immersed in this solution was severely wilted when examined 24 hours later. The toxic substance, therefore, was precipitated with the proteins.

Seventeen-day-old cultures of *Phytophthora nicotianae,* grown on Richards' solution, were used to test the effect of temperature on the stability of the toxin. One portion was stored for 48 hours at 34° F. Another portion was boiled for 5 minutes. After the solutions had regained room temperature, they were filtered through a Büchner funnel, and a healthy tobacco plant was placed in each. One 19-day-old culture and a flask of noninoculated Richards' solution, in which plants were placed, served as controls. The plant with roots immersed in the boiled filtrate wilted in 15 minutes, while 25 minutes were required to wilt the plant in the filtrate that had been subjected to a temperature of 34° F. The plant in the 19-day-old culture wilted badly in 15 minutes, and the one placed in the noninoculated Richards' solution remained normal. This evidence would seem to indicate that the toxic substance is not affected by temperatures within the range 34° to 212° F.

In testing for volatility of the toxin, a 20-day-old flask culture on Richards' solution was distilled for 30 minutes, and the gases that came off were collected in distilled water. They were colorless and of a pungent, disagreeable odor. After cooling, a plant was placed in the distillate and another in the liquid remaining in the distilling flask. The latter wilted severely in 8 minutes, while the plant in the distillate remained turgid, showing that the toxin is nonvolatile.

ACID PRODUCTION BY THE ORGANISM

A quantity of potato broth, which had an initial reaction of pH 6.5, was used as the nutrient solution in this experiment. Portions of this medium were adjusted with HCl and NaOH to concentrations of 4.0, 4.8, 7.2, 8.2, and 8.8, and 1 portion was left unadjusted. After tubing and autoclaving, the solutions were inoculated with *Phytophthora nicotianae*. Colorimetric tests of changes in reaction were made at intervals, as shown in the following table:

TABLE 1.—*Changes in acidity of cultures of Phytophthora nicotianae*

Initial reaction	Reaction after			
	4 days	6 days	8 days	9 days
4.0	4.0	3.6	3.4	3.4
4.8	4.6	4.6	4.4	4.2
6.5	5.6	5.0	4.6	4.6
7.2	6.2	5.6	5.0	4.6
8.2	6.8	5.6	5.0	4.6
8.8	7.8	7.2	5.4	5.0

The cultures the initial concentration of which were pH 4.8 and 6.5 made the most rapid growth. This accords with the findings of Tisdale and Kelley (21), who state that pH concentrations of 4.4 to 5.1 are most favorable for growth.

In view of the fact that many fungi are known to produce oxalic acid, it seemed desirable to test for the presence of oxalates. When a mixture of the filtrate and concentrated sulphuric acid was heated and the gases given off were passed through baryta water, there was no evidence of the formation of barium carbonate. Also, when the filtrate was neutralized with NaOH and a solution of $CaCl_2$ was added, no precipitate of calcium oxalate was formed. Both tests show, therefore, that the acids formed in these cultures are not oxalic.

INFLUENCE OF PH CONCENTRATION ON WILTING

In view of the fact that plants wilted when placed in filtrates of the fungus, it seemed desirable to determine if the pH concentration of the filtrate was a cause of this wilting. The fungus was allowed to grow in half-strength Richards' solution for 17 days. The mycelial mat was removed, and the solution filtered through a Büchner funnel. After filtration, portions of the nutrient solution were adjusted to pH concentrations of 4.4 and 8.6. Healthy tobacco plants were placed in flasks containing these adjusted filtrates. Examination after 30 hours showed both plants severely

wilted, while those placed in flasks of noninoculated Richards' solution, at the same concentrations, showed no wilting in a corresponding period. This is interpreted to show that neither organic acids produced by the pathogen nor the pH concentration of the media employed is responsible for the wilting of tobacco.

UTILIZATION OF CARBOHYDRATES

In unpublished data Carlos[2] has shown that the black-shank organism digests the middle lamella and the secondary cellulose membranes. The secretion of enzymes other than those responsible for these phenomena was tested by the use of carbohydrate media. Standard starch-agar plates were poured, inoculated with *Phytophthora nicotianae*, and allowed to stand for 3 days. When the plates were flooded with iodine, a colorless area appeared surrounding each colony, demonstrating the digestion of starch by the enzyme diastase (amylase) secreted by the fungus.

To study the possibility of the digestion of sugars by *Phytophthora nicotianae*, broths consisting of 2 per cent peptone and 2 per cent of dextrose, maltose, sucrose, or lactose, were made up and inoculated. One portion consisting of 2 per cent peptone alone was inoculated to serve as a check. The digestion of these sugars was determined colorimetrically by the change in hydrogen-ion concentration.

In dextrose and maltose, a change in reaction of 1.0 was produced during a 5-day period, and in sucrose a decrease of 0.8, indicating that these sugars are readily digested by the fungus. The digestion of dextrose, maltose, and sucrose necessitates that the fungus produce zymase, maltase, and invertase. In lactose and in peptone broth, on the other hand, the change within the given period amounted to only 0.2.

SUMMARY

The rapid wilting and collapse of tobacco plants infected with *Phytophthora nicotianae* are due to a toxin. This toxin may be extracted from black-shank lesions and is also present in cultures in which the organism has been grown. This toxin, which is thermostable and nonvolatile, is probably protein in nature.

Acids other than oxalic are produced by the black-shank fungus during the decomposition of tissues and in culture media. These acids do not account for the wilting, however.

Phytophthora nicotianae secretes enzymes that enable it to utilize the middle lamella, the secondary membranes, starch, sucrose, dextrose, and maltose.

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FIELD OBSERVATIONS OF NEEDLE RUSTS OF SPRUCE IN MINNESOTA

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In the summer of 1927 a needle rust of spruce appeared in epidemic form in northwestern Minnesota. The regional distribution of the disease in the State corresponded to the range of the swamp evergreen heath plants, Labrador tea, *Ledum groenlandicum* Oeder; leatherleaf, *Chamaedaphne calyculata* (L.) Moench; and bog rosemary, *Andromeda glaucophylla* Link. These plants became heavily infected with the alternate stages of the causal organisms later in the summer. The infected spruce was the cause of considerable concern to foresters, nurserymen, and owners of ornamental spruce plantings because epidemics of a similar nature had been infrequent, and little information was available on the nature and extent of the resulting damage. The prevalence of the disease during 1927 on ornamental as well as on native spruce, growing under natural conditions, afforded an excellent opportunity to add to the meager information concerning such epidemic attacks.

NOTES ON INFECTION

Immature aecia were first reported on spruce on July 14. Shedding of the spores began towards the latter part of July and continued for 3 weeks in August. Although infection was limited to the current year's growth, aecial production, especially in the case of native black and Colorado blue spruce, growing in areas of heavy attack, was so abundant that the foliage in mass was yellow. The aecial pustules occurred in irregular rows, sometimes definitely 2, on the under side of the needle, and varied in number from 3 to 9 but were usually 5 to 6. Trees of all ages were attacked, infection being heaviest in the leaders and upper portions of small trees under 8 ft. in height. In the larger trees, infected needles were confined for the most part to the lower portions of the crown. The relative susceptibility of several species of spruce growing side by side in forest nurseries was observed to be as follows: susceptible—black spruce, *Picea mariana* (Miller) Britton, Sterns, and Poggenberg, and Colorado blue spruce, *P. pungens* Engelmann; moderately susceptible—white spruce, *P. glauca* (Moench) Voss; resistant—Black Hills spruce, *P. glauca albertiana* (S. Brown) Rehder, and Norway spruce, *P. excelsa* Link. No attempt was made to substantiate these field observations on susceptibility by conducting controlled inoculation experiments on spruce.

The heath plants, leatherleaf, Labrador tea, and bog rosemary were usually found in the immediate vicinity of infected spruce, although in the

case of one nursery that contained rusted trees, the nearest plants found were ½ mile distant. Examinations were made of these plants at intervals during the month of August. The small red to yellow uredinial pustules were first noticed on August 16 on leatherleaf growing in the vicinity of heavily rusted black spruce. By the latter part of August this stage had become prevalent on both leatherleaf and Labrador tea, being hypophyllous in the case of the former and predominantly hypophyllous but occasionally epiphyllous on Labrador tea. Comparatively little infection was found on bog rosemary, even where heavily rusted plants of the other 2 species were in close proximity. Uredinial production continued through the months of August and September and was heaviest on those plants growing close to or under diseased spruce. Infection was most common on the thin small leaves that had not yet become leathery and were relatively free of tomentum. An examination of infected areas made in March, 1928, revealed only uredinia on old leaves. Clinton[1] reported having found uredinia in January on living old plants and believed them to be capable of overwintering.

NATURE AND EXTENT OF INJURY TO SPRUCE

Infected needles were being shed even before the aecia had ceased to liberate spores. Where heavy infection occurred, as on small trees and in the lower portions of the crown of large trees of black and Colorado blue spruce, it was estimated that from ¼ to ¾ of the current year's needles were lost by the end of the summer. Defoliation was slight in the case of white spruce and was of little or no consequence on Black Hills and Norway spruce. Whether such defoliation as occurred on black and Colorado blue spruce retarded growth or resulted in permanent injury was not established definitely. Reports received in 1928 from foresters and nurserymen indicated that the infected trees had recovered to the extent that little evidence remained of the heavy attack of the previous year. No killing or apparent deformation of the infected trees had occurred, although it was reported by several that growth had been retarded slightly. The effect of several successive years of defoliation could not be determined because only light attacks of the rust occurred in 1928 and 1929. It is probable that successive epidemics would result in pronounced retardation of growth, and possibly in permanent injury to the heavily infected plants.

The only known previous reports of injury to spruce caused by the needle-rust fungi are those of Clinton, who states that needles infected with *Melampsoropsis cassandrae* appeared as though they would be shed prematurely; and of Fraser, who describes killing of young plants by heavy pycnial infection in inoculation experiments with one of the rust fungi.

[1] Clinton, G. P. Heteroecious rusts of Connecticut having a Peridermium for their aecial stage. Conn. Agr. Expt. Sta. Ann. Rpt. 1907–1908: 369–396. 1908.

CAUSAL ORGANISMS

Three closely related and very similar needle-rust fungi have been described as commonly occurring on spruce, namely; *Melampsoropsis abietina* (Alb. and Schw.) Arthur, *M. ledicola* (Peck) Arthur, and *M. cassandrae* (Peck and Clinton) Arthur. These have as their alternate hosts evergreen swamp plants belonging to the heath family; principally leatherleaf, Labrador tea, and bog rosemary. The host relationships of 2 of these, *M. abietina* and *M. ledicola*, were established by Fraser[2] in 1910 and 1911 through studies conducted under both field and controlled conditions. The relation between *Peridermium consimile* and *M. cassandrae* was shown by Clinton[3] in 1908, and this work was later substantiated by Fraser. The aecial stages of all 3 of these organisms appear as needle rusts on spruce and very similar morphologically and, therefore, difficult to differentiate. The uredinial stages are more readily distinguished, those of *M. abietina* and *M. ledicola* being hypophyllous and epiphyllous forms, respectively, on Labrador tea; while the uredinial stage of *M. cassandrae* occurs as a hypophyllous form on leatherleaf.

In an effort to establish the identity and relative prevalence of the organisms concerned in the epidemic of 1927, examinations were made of the aecial and uredinial stages, both in the field and in the laboratory. The distinguishable differences in the aecial stages were found to be insufficient to permit a determination of the causal organisms on spruce. Since the uredinial stages of all 3 organisms were found on the heath plants, there was evidence that all were concerned to some extent. The heaviest infection occurred on leatherleaf, which suggested that *Melampsoropsis cassandrae* was at least common and probably most prevalent. On Labrador tea, the uredinial sori were predominantly hypophyllous, indicating a greater prevalence of *M. abietina* than of *M. ledicola*.

A number of inoculations of leatherleaf and Labrador tea were made in the field and in the greenhouse with the purpose of obtaining supplementary evidence on the relative prevalence of the several rust fungi. A search for a rust-free area was unsuccessful; consequently, plants growing in areas of light infection had to be used. In the field inoculations, 10 apparently noninfected plants of each species growing *in situ* were sprayed with fresh aecial material collected from black and Colorado blue spruce. Within 20 days all 20 plants showed infection, the uredinial sori being hypophyllous in all cases and more abundant on leatherleaf than on Labrador tea. Noninoculated plants in the immediate vicinity of the sprayed ones were either free of infection or showed light attacks only on the lower sides of the leaves.

[2] Fraser, W. P. Cultures of some heteroecious rusts. Mycologia 3: 67–74. 1911.
[3] *Loc. cit.* See footnote 1.

For the greenhouse studies, 40 plants of each species, together with the moss and substratum in which they were growing, were taken to the University Farm and potted immediately. Thirty plants of each species were sprayed with aecial material collected from infected spruce in the field, and 10 were kept as controls. A number of the plants soon showed evidences of loss of vigor as a result of transplanting, and several had withered before the incubation period of 21 days was completed. Of the 60 inoculated plants, 7 leatherleaf and 2 Labrador tea developed uredinial pustules on the lower sides of the leaves. Of the controls, all except 1 leatherleaf remained free of rust, infection in this 1 case probably having occurred while the plant grew in the field.

The meager infection results obtained in the inoculation studies permit no definite conclusions. It is interesting to note, however, that they tend to strengthen field observations, which indicated that *Melampsoropsis cassandrae* was very common on spruce and that *M. ledicola* was of little significance.

SUMMARY

A needle rust of spruce appeared in epidemic form in Minnesota during the summer of 1927. The relative susceptibility of spruce, growing in forest nurseries, was observed to be as follows: susceptible, black and Colorado blue spruce; moderately susceptible, white spruce; resistant, Norway and Black Hills spruce. Defoliation in the case of heavily infected plants amounted to from $\frac{1}{4}$ to $\frac{3}{4}$ of the current year's needles. Reports received in 1928 indicated that no killing or permanent deformation of the infected trees had resulted.

Field and laboratory examinations of the uredinial stages of the causal organisms on heath plants indicated *Melampsoropsis cassandrae* to be very common; *M. abietina* to be fairly common; and *M. ledicola* to be of little significance. Inoculation studies on leatherleaf and Labrador tea tended to strengthen the above conclusions as to the relative prevalence of the several fungi.

CONSISTENT MUTATION OF HELMINTHOSPORIUM SATIVUM ON A NO-NITROGEN MEDIUM

GLENN E. PAXTON

(Accepted for publication August 22, 1932)

Numerous workers have studied environmental factors involved in mutation of fungi. Stevens (5), Christensen (1), Leonian (3), Mitra (4), and others have shown that the type and amount of medium greatly influences the frequency of sector formation. Christensen (2) has shown certain temperatures to be more favorable than others.

The effect of nutrition on mutation has been studied in preliminary experiments, and evidence is presented here that mutations may be produced consistently in *Helminthosporium sativum* P., K., & B. on a standard medium.

The culture of *Helminthosporium sativum* studied by the writer was isolated from a barley leaf showing typical spot-blotch lesions that had been collected at Davis, California, in 1931. The plates were incubated at room temperature (18–25° C.) and examined after 2 to 3 weeks.

Czapek's medium was used in order to obtain the normal growth of the fungus. It was made up as follows: 0.5 gm. $MgSO_4$, 0.5 gm. KCl, 1 gm. KH_2PO_4, 0.01 gm. $FeSO_4$, 2 gm. $NaNO_3$, 30 gm. sucrose, and 20 gm. of agar in 1,000 cc. distilled water.

It was found that mutations or sectors occurred consistently when the sodium nitrate was omitted from Czapek's medium. This will be designated as the minus-N medium. Different depths of medium were used in Petri dishes with no difference in results. The usual amount employed was 20 cc. Single-spore or mass transfers on this medium produced similar colonies.

The results of 5 series of tests may be summarized as follows: On minus-N agar all of the 47 colonies from mass transfers and all the 91 single-spore colonies produced sectors, whereas among 44 colonies from mass transfers on Czapek's agar 3 produced sectors; among 103 colonies from single spores only 9 produced sectors.

Small white tufts of sterile mycelium were often produced on the colonies, but only the fan-shape sectors were taken into consideration in the results. The number of sectors per plate on the minus-N medium varied from 2 to 12, with an average of 5. The sectors grew faster than the remainder of the colony and were usually whiter and sporulated more sparingly, as shown in figure 1, A.

Sectors occurred occasionally on Czapek's agar, but all except one were unlike those that occurred on the minus-N medium, in that most of them were essentially sterile white mycelium. This difference was especially noticeable when the mutants were grown for comparison on Czapek's agar.

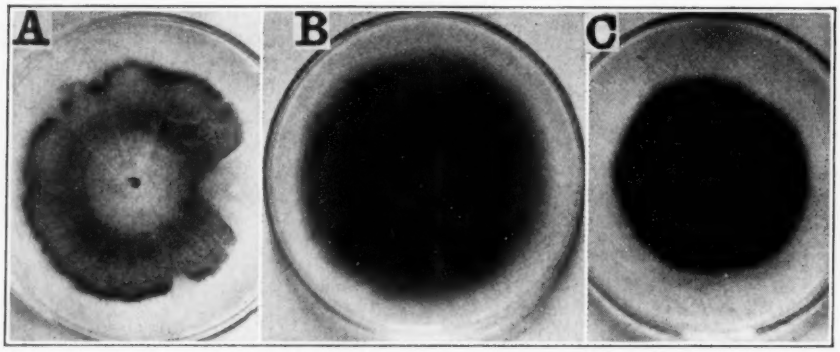

FIG. 1. A. Three-week culture of *Helminthosporium sativum* on minus-N medium showing sectors. B. Fifteen-day culture of the mutant growing on Czapek's agar. C. Fifteen-day culture of the original fungus growing on Czapek's agar.

The sectors that occurred on the minus-N medium were of many types, one of which invariably occurred on every plate. This type of mutant was studied in detail and is shown (Fig. 1, B) growing on Czapek's agar, in comparison with the original fungus on the same medium (Fig. 1, C). As compared with the normal, the mycelium of the mutant was darker, grew faster, the sporulation was heavier, and much more variation was evident in the length and shape of the spores. The spores of the mutant were darker brown, shorter, and more rounded at the ends. Five hundred spores averaged 55×24 μ, while the spores of the normal fungus averaged 72×22 μ.

Mass and single-spore transfers were made from the minus-N to Czapek's agar from the sectors and from the normal region between the sectors of the colonies. Change had occurred only in the transfers from the sectors.

When the spores were taken from the normal region of the colony and again grown on the minus-N medium, sectors occurred in every trial. Colonies taken from the sectors and grown on the minus-N medium have sectored only twice in about 100 trials. No sectoring of the mutant has occurred during 5 generations on Czapek's agar. Colonies from some of the other types of sectors not considered in this paper have, however, sectored still further on the minus-N medium and on Czapek's agar. Some have shown a partial reversion to the normal fungus.

The effect of the 2 media on anastomosis of hyphae was tested by growing the normal fungus on slides covered with a thin layer of agar. There was abundant hyphal fusion on both the Czapek's and the minus-N agars and no noticeable difference was observed. The same was true in observations made on many colonies growing in Petri dishes.

It has been found that the addition of $\frac{1}{2}$ gm. of $NaNO_3$ per liter to the minus-N medium was sufficient to prevent most of the sector formation. When both sucrose and nitrogen were omitted from the Czapek's medium no

sectors were formed. Among 20 cultures grown on minus-N agar with only 10 gm. of sucrose per liter, and 20 cultures grown on minus-N agar with 100 gm. of sucrose per liter, sector formation was not interfered with in any plate. In 10 cultures of the normal fungus, KNO_3 was substituted for $NaNO_3$ in Czapek's agar and no difference in results was obtained. The omission of sodium was apparently not a factor in sector formation.

Twenty colonies, each, of 2 other forms of *Helminthosporium sativum* were grown on the minus-N medium and every one produced sectors.

Other fungi, such as Penicillium, Alternaria, and a Macrosporium were grown on 20 plates, each of the minus-N agar, but no sectors were produced.

Mutation in *Helminthosporium sativum* appeared to be related to nutrition. Lack of nitrogen seemed to be an important factor in causing consistent mutation. If nitrates were present, not only were decidedly fewer sectors formed, but these were different in morphology. A slower growth rate, increased time factor, and accumulation of by-products in the absence of nitrogen may explain partly the phenomenon of sector formation. Sucrose also plays an important part because, when this was omitted, no sectoring occurred. Depth of medium did not affect either the number or type of sectors. Since anastomosis of hyphae was abundant on both media, it is improbable that it has any effect in causing sector formation.

SUMMARY

On Czapek's agar, *Helminthosporium sativum* produced only occasional sectors, while all colonies on Czapek's agar, containing no nitrogen, produced sectors, one type of which occurred on every culture and was distinctly different from the original colony. Subplants from this mutant have shown no tendency to revert to the original, even in the fifth generation. On Czapek's agar, lacking sucrose as well as nitrogen, no sectors were produced.

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PHYTOPATHOLOGICAL NOTES

Three New Hosts for Tylenchus dipsaci, the Bulb or Stem Nema.—Bulbs of *Colchicum speciosum album* (Hort.), imported from Haarlem, Holland, and intercepted at the Inspection House of the Bureau of Plant Quarantine in Washington, D. C., August, 1932, were found to be infested with *T. dipsaci* (Kühn, 1858) Bastian, 1865. As far as we are aware, this is the first time that this parasite has been reported from a Colchicum. About 20 per cent of the bulbs of the shipment were affected. Judging from our observations, the disease must be very detrimental to the Colchicum bulbs. (Fig. 1, A.) The symptoms somewhat resemble those produced by the same did not revive, but were otherwise excellently preserved.—G. STEINER and parasite in bulbous irises. In every instance observed the infestation began at the base of the bulb, destroying the basal or root plate. The first signs of the disease are yellowish or brown streaks, which gradually spread out on the surface but also penetrate the bulb more and more. To properly see these symptoms, the brown cover scale must be loosened as shown in figure 1, A. A more advanced stage of the disease presents areas of a grayish color penetrating the bulb quite deeply. It consists of destroyed tissues that soon completely decay and then form a black powdery mass. The progress of the disease is shown in stages reading from left to right, (Fig. 1, A).

In the fall of 1932 shipments of *Chionodoxa luciliae* Boiss. bulbs, intercepted in New York City, Philadelphia, and Detroit, and all originating from Holland, were found infested with *Tylenchus dipsaci.* This seems to be the first record of this nemic pest in Chionodoxa. The disease symptoms occur externally, as well as internally, since there are produced both surface scales and internal brown rings analogous to the brown-ring symptoms of narcissus. (Fig. 1, B.) The affected surface scales are shriveled and exhibit a brown to black discoloration.

In January, 1933, two herbarium leaves of foxglove, *Digitalis purpurea* L., from the Elm City Nursery, New Haven, Connecticut, collected in June, 1916, were received from G. P. Clinton of the Connecticut Agricultural Experiment Station. The leaves (Fig. 1, C) showed spots in which large numbers of *Tylenchus dipsaci* were found. *D. purpurea* was not known to be a host of this nemic pest. The symptons are somewhat unusual for this nema, being more of the type produced in certain hosts by *Aphelenchoides fragariae.* Probably the parasites invaded the leaves under wet conditions from the under surface, entering through the stomata all over the leaves and forming thereby numerous infection centers simultaneously. The *T. dipsaci* specimens, having been in dry herbarium preservation for 16½ years, EDNA M. BUHRER, Bureau of Plant Industry, U. S. Department of Agriculture, Washington, D. C.

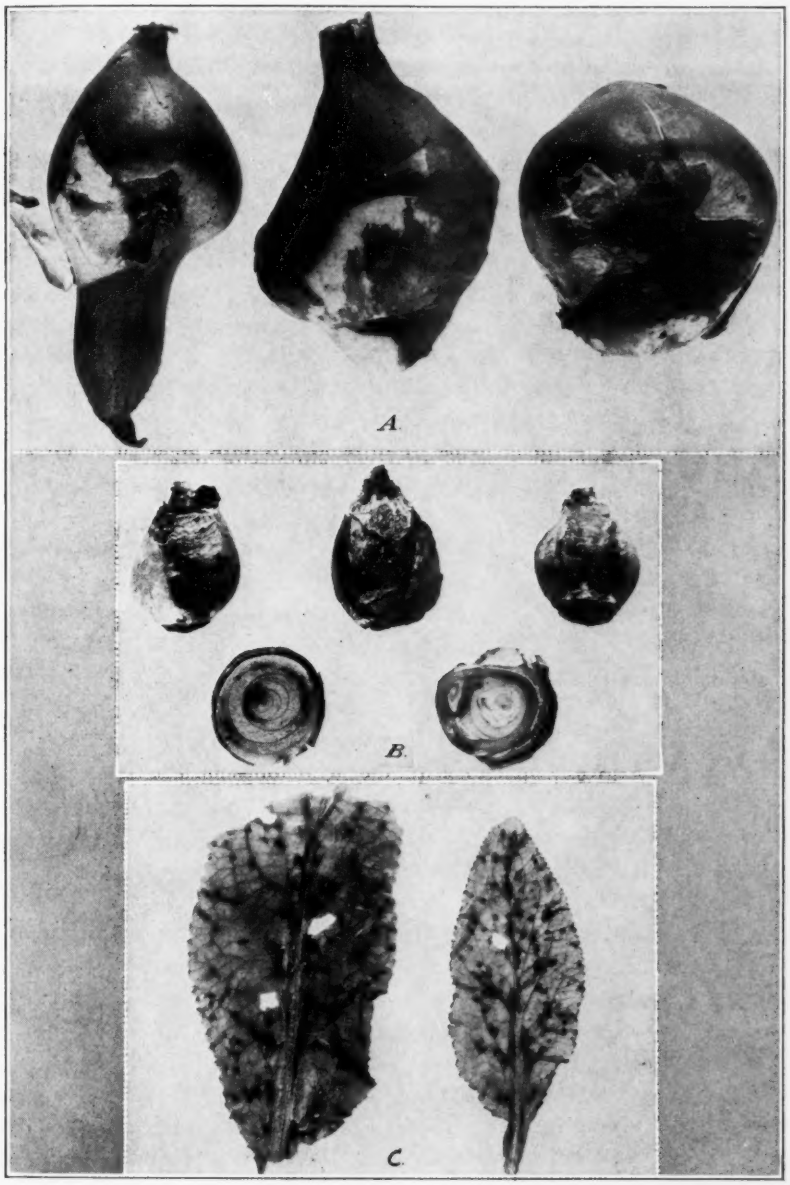

FIG. 1. A. *Colchicum speciosum album* bulbs infested with *Tylenchus dipsaci*. Progress of the disease shown in stages reading from left to right. B. *Chiondoxa luciliae* bulbs infested with *Tylenchus dipsaci* showing external and internal areas of infestation. C. *Digitalis purpurea* leaves infested with *Tylenchus dipsaci*.

Unusual Disease Symptoms Produced by Aphelenchoides fragariae.—Recently, some *Chrysanthemum hortorum* (Hort.) plants, which proved to be infested by *Aphelenchoides fragariae* (Ritzema Bos 1891) Steiner and Buhrer 1932,[1] were received from a nursery in Ohio. The disease symptoms were not those commonly observed on chrysanthemums when attacked by this nematode, *i.e.*, brown or yellow spotting of the leaves. Instead, the plants were a full and healthy green but exhibited defective leaf spread, some leaves being almost filiform, reduced, and abnormally crinkled, the leaf stem often swollen (Fig. 1, *a*). In some instances stems and leaves were dwarfed, crinkled, and bunched (Fig. 1, *a*, *d*), producing, with a shortened swollen stem, what might be called the "cauliflower" type of disease, not previously observed in chrysanthemums (Fig. 1, *b*). Stems may even be blind (Fig. 1, *c*). The affected plants were apparently of luxuriant, fast growth, the tissue being sappy and rather soft, a fact that may account for the unusual symptoms. *A. fragariae* is known to produce cauliflower symptoms in strawberry plants, the explanation offered in this case being similar to that just mentioned. Various host plants of the bulb or stem nema (*Tylenchus dipsaci*), if in luxuriant, sappy growth, also react to such a nemic infestation by production of swollen curved stems and crinkled leaves (cauliflower type), whereas under dry conditions and slower growth no such symptoms are developed.—G. STEINER and EDNA M. BUHRER, Bureau of Plant Industry, U. S. Department of Agriculture, Washington, D. C.

A Potato Disease.—Potatoes, both in greenhouse and field at Ithaca, New York, became affected in late August, 1932, with a serious disease, which had the general appearance of top necrosis (acronecrosis) and was so considered for some weeks. The disease was observed first in species hybrids of domestic by *Solanum demissum, antipoviczii,* etc., from Mexico but soon spread to a number of domestic varieties, Rural New Yorker, Green Mountain, Katahdin, Triumph, and others, as well as to several tuberous and nontuberous species of Solanum from Mexico, also to *Physalis* spp., to *Nicandra physaloides,* to *Datura stramonium,* to pepper (*Capsicum* sp.), and to tomato (Lycopersicum).

Symptoms appear first in the apical leaflets. The leaflets begin to cup downward and show numerous darkish areas on the underside. The tips of shoots begin to turn brown and from day to day become browner and very brittle. In the meantime the undersides of the leaflets assume a glassy appearance followed by a bronzing of the whole area. The leaf petiole becomes brittle and the leaf breaks off with a touch. Axillary shoots pass through these stages rapidly and die completely. The stalks and fully

[1] Synonyms of this form are: *Aphelenchus subtenuis* Cobb 1926, *A. olesistus* Ritzema Bos 1893, and *A. ritzema-bosi* Schwartz 1911.

FIG. 1. *Chrysanthemum hortorum* infested with *Aphelenchoides fragariae*. Outstanding symptoms marked with arrows, as explained in text.

matured leaves may remain green for a long time or they may die rather promptly, apparently depending on the age of the plant when first attacked. First-year potato seedlings usually are killed outright. The long stolons of Mexican species and of many of the hybrids are very brittle on affected plants as compared with healthy ones. Tuber formation is checked as soon as the disease is contracted.

The disease is readily communicated by grafting and by leaf mutilation with diseased material so that in very many respects it has the characters of a virus disease. Mention of the disease in a session of The American Phytopathological Society elicited the information that a mite probably is responsible for the trouble, and this proves to be the case. The creatures are microscopic in size and scarcely seem abundant enough on the foliage to account for the profound changes induced in the plants. The mite involved is of the genus Tarsonemus, but the specific identity is a matter for further study that is being done by Wm. Blauvelt, of Cornell University. Mr. Blauvelt's familiarity with the Cyclamen mite permits him to say definitely that it is a different species.

With the exception of minor variations, the disease corresponds with the brief description of Carpenter[1] concerning an outbreak of mites in Hawaii and with that of Mann *et al.*[2] of an outbreak in India. The latter authors found the disease a very serious one in the "summer" crop of potatoes in the whole Poona district of India, and Kulkarni[3] later found peppers over wide areas in India severely injured by the same organism.

The mites yield to fumigation with naphthalene.[4] It is a relatively simple matter to rid a greenhouse of the pests, but their occurrence in the field is a more serious matter. Lime sulphur solution and sulphur dust have been used effectively in fields of India. No outbreak of this or a similar pest in the field seems to have been recorded for continental United States of America. It may be that the Ithaca outbreak of 1932 is sporadic, but it would be unwise to minimize the possible danger. Every effort will be made to avoid further trouble in 1933.—DONALD REDDICK, Cornell University, Ithaca, New York.

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BOOK REVIEW

Root Nodule Bacteria and Leguminous Plants. By E. B. Fred, I. L. Baldwin, and Elizabeth McCoy. 343 pp. University of Wisconsin Studies in Science No. 5, Madison, Wisconsin. 1932.

While this book is primarily a review of the entire literature pertaining to root nodules and root-nodule bacteria of leguminous plants, it is thoroughly interspersed with authoritative opinions and deductions. The authors have brought to light from available history material that it is rarely the pleasure of most of us to see. Reference to the use of legumes as crops and food is carried back about 6,000 years, and evidence is presented to show that the seeds of the lentil, a legume, were used by the builders of the pyramids. The writings of Cato in the 2nd century B. C. give one of the first indications of an appreciation for the leguminous plant as a soil benefactor. The necessarily fragmentary ancient history of legumes is followed by a discussion of their use in New World agriculture from the time of discovery to the present period and also includes some of the recently acquired archaeological data from Mayan and Pre-Incan explorations. This first chapter chronicles the classical experiments with legume bacteria by scientists of the 19th century, culminating in the work of Hellriegel, to whom credit for substantial progress in explaining nitrogen fixation by legumes is given.

Legumes are considered botanically in the 2nd chapter, and here, as well as in the 12th chapter, they are discussed from an economic standpoint. Covering a period from 1587, a summary is given of the conceptions of various observers of the nature and functions of root nodules of legumes. Space is also devoted to nonnodule-bearing legumes, to nodule-bearing nonlegumes, and to the normal leaf growths that harbor presumably essential bacteria. A continuance of the work on nodules is found in chapter 9 where their life history is carried through the infection thread stage, nuclear alteration, and the digestion of the bacteria in the cells.

Starting with chapter 4, methods of isolating and differentiating legume bacteria are presented, followed by a discussion of the known life history of the organism and its responses to environmental influences such as temperature, light, aeration, and composition of the substratum.

Of interest to pathologists is the part of chapter 10 concerning variations in physiological efficiency among nodule organisms that amounts to parasitism at times but is usually of a beneficial nature. The work on nitrogen fixation by a combination of the plant and its organism and by the organism alone brings the comment "that perhaps the effort to divorce the rôles

of the plant and bacteria in the process of nitrogen fixation is fundamentally wrong.''

The literature on the influences that affect nodule production leads the authors to believe that there is much yet to be learned regarding environment in relation to nodule production. The last chapter is concerned with the practical application of nodule bacteria to soils and seeds; the various methods of transferring field soil are outlined; and an interesting history of the development of the artificial or pure-culture business in Europe and the United States is given with considerable detailed reference to patents issued in this connection. An appendix includes specific directions for the use of artificially prepared legume-bacteria cultures, a list of sources from which inoculating material of this type may be obtained, data on the extent of the distribution of such cultures, and the names of governmental agencies concerned in the regulation of the trade together with the laws through which they operate.

In addition to a wealth of information most of which it is not possible to mention here, the book contains many illustrations. It will be a great asset to scientific workers and, in view of its practical bent, should serve as an informational handbook to those interested in soil bacteriology from a commercial standpoint.

A bibliography of 1,058 references follows the appendix.—LEWIS T. LEONARD, Bureau of Chemistry and Soils, U. S. Department of Agriculture.